# DEVELOPMENT OF TOPICAL GELS CONTAINING CHITOSAN-AND PEG-BASED MICROPARTICLES LOADED WITH DUAL DRUGS (DICLOFENAC SODIUM AND SULFAMETHOXAZOLE) FOR BACTERIAL SKIN INFECTIONS

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**CHENNAI - 600 032**

**In partial fulfillment of the requirements for the award of the Degree of**

**MASTER OF PHARMACY**

**IN**

**BRANCH- I - PHARMACEUTICS**


**Submitted by**

**Ms. J. Joslin Jenishiya**

**REGISTRATION No.**

261610352

**Under the guidance of**

**Mr. T. RAJA SEKHARAN, M.Pharm.,**

**Assistant Professor**

**Department of Pharmaceutics**




**SANKARALINGAM BHUVANESWARI COLLEGE OF PHARMACY,**

**ANAIKUTTAM, SIVAKASI-626130**


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**Mr. T. Raja Sekharan., M.Pharm.,**

Assistant Professor,

Department of Pharmaceutics,

Sankaralingam Bhuvaneswari College of Pharmacy,

Sivakasi-626130,

Tamil Nadu State, India**.**

## CERTIFICATE BY THE GUIDE

This is to certify that the dissertation entitled, **"DEVELOPMENT OF TOPICAL GELS CONTAINING CHITOSAN-AND PEG-BASED MICROPARTICLES LOADED WITH DUAL DRUGS (DICLOFENAC SODIUM AND SULFAMETHOXAZOLE) FOR BACTERIAL SKIN INFECTIONS",** submitted by **Ms. J. Joslin Jenishiya (Reg. No. 261610352)** to **The Tamil Nadu Dr. M.G.R Medical University, Chennai,** for the award of degree of **"MASTER OF PHARMACY IN PHARMACEUTICS"** is a bonafide research work carried out by her in the **Department of Pharmaceutics, Sankaralingam Bhuvaneswari College of Pharmacy, Sivakasi,** under my guidance and supervision. The content of this dissertation in full or in parts have not been submitted to any other Institute or University for the award of any degree or diploma.

Place: Sivakasi **Mr.T. Raja Sekharan, M.pharm.,**

Date:

**Dr. M. Rajesh, M.Pharm., Ph.D.,**

Professor and Head,

Department of Pharmaceutics,

Sankaralingam Bhuvaneswari College of Pharmacy,

Sivakasi-626130,

Tamil Nadu**.**

## HEAD OF THE DEPARTMENT CERTIFICATE

This is to certify that the dissertation entitled, **"DEVELOPMENT OF TOPICAL GELS CONTAINING CHITOSAN-AND PEG-BASED MICROPARTICLES LOADED WITH DUAL DRUGS (DICLOFENAC SODIUM AND SULFAMETHOXAZOLE) FOR BACTERIAL SKIN INFECTIONS",** submitted by **Ms. J. Joslin Jenishiya (Reg. No. 261610352)** to **The Tamil Nadu Dr. M.G.R Medical University, Chennai,** for the award of degree of **"MASTER OF PHARMACY IN PHARMACEUTICS"** is a bonafide research work carried out by her in the **Department of Pharmaceutics, Sankaralingam Bhuvaneswari College of Pharmacy, Sivakasi,** under the guidance and supervision of **T. Raja Sekharan**. The content of this dissertation in full or in parts have not been submitted to any other Institute or University for the award of any degree or diploma.

Place: Sivakasi **Dr. M. Rajesh, M.Pharm., Ph.D.,**

Date:

**Dr. P.Solairaj, M.Pharm., Ph.D.,**

Principal,

Sankaralingam Bhuvaneswari College of Pharmacy,

Sivakasi-626130,

Tamil Nadu**.**

## ENDROSEMENT BY THE PRINCIPAL

This is to certify that the dissertation entitled, **"DEVELOPMENT OF TOPICAL GELS CONTAINING CHITOSAN-AND PEG-BASED MICROPARTICLES LOADED WITH DUAL DRUGS (DICLOFENAC SODIUM AND SULFAMETHOXAZOLE) FOR BACTERIAL SKIN INFECTIONS",** submitted by **Ms. J. Joslin Jenishiya (Reg. No. 261610352)** to **The Tamil Nadu Dr. M.G.R Medical University, Chennai,** for the award of degree of **"MASTER OF PHARMACY IN PHARMACEUTICS"** is a bonafide research work carried out by her in the **Department of Pharmaceutics, Sankaralingam Bhuvaneswari College of Pharmacy, Sivakasi,** under the guidance and supervision of **T. Raja Sekharan**. The content of this dissertation in full or in parts have not been submitted to any other Institute or University for the award of any degree or diploma.

Place: Sivakasi **Dr. P. Solairaj, M.Pharm., Ph.D.,**

Date:

# EVALUATION CERTIFICATE

This is to certify that the dissertation entitled, **"DEVELOPMENT OF TOPICAL GELS CONTAINING CHITOSAN-AND PEG-BASED MICROPARTICLES LOADED WITH DUAL DRUGS (DICLOFENAC SODIUM AND SULFAMETHOXAZOLE) FOR BACTERIAL SKIN INFECTIONS",** submitted by **Ms. J. Joslin Jenishiya (Reg. No. 261610352)** to **The Tamil Nadu Dr. M.G.R Medical University, Chennai,** for the award of degree of **"MASTER OF PHARMACY IN PHARMACEUTICS"** was evaluated by us during the academic year 2017-2017.

**INTERNAL EXAMINAR** **EXTERNAL EXAMINAR**

Date: Date:

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*Place: Sivakasi.*

**Ms. J. Joslin Jenishiya**

***(Reg.No. 261610352)***

*Date:*

# DEDICATED TO ALMIGHTY GOD, MY BELOVED PARENTS AND GUIDE

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## ABBREVATIONS

| | |
|---|---|
| UV | Ultra Violet |
| IR | Infra Red |
| FTIR | Fourier Transform Infrared Spectroscopy |
| PhEUR | European Pharmacopoeia |
| J.P | Japanese Pharmacopoeia |
| U.S.P | United States Pharmacopoeia |
| SD | Standard Deviation |
| rpm | Rotation Per Minute |
| nm | Nanometre |
| hrs | Hours |
| min | Minutes |
| sec | Seconds |
| gm | Gram |
| mg | Milligram |
| ml | Millilitre |
| mm | Millimetre |
| cm | Centimetre |
| µm | Micrometre |
| µg/ml | Microgram per milliliter |
| gm/ml | Gram per milliliter |
| mg/ml | Milligram per milliliter |
| $gm/cm^3$ | Gram per centimetre cube |
| $Kg/cm^2$ | Kilogram per centimetre square |
| w/w | Weight per weight |
| w/v | Weight per volume |
| % | Percentage |
| $^oC$ | Degree Celsius |

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# *Introduction*

## INTRODUCTION- MICROPARTICLES

Oral drug administration is by far the most preferable route for taking medications. However, their short circulating half life and restricted absorption via a defined segment of intestine limits the therapeutic potential of many drugs. Such a pharmacokinetic limitation leads in many cases to frequent dosing of medication to achieve therapeutic effect. This results in pill burden and consequently, patient complains. Rational approach to enhance bioavailability and improve pharmacokinetic and pharmacodynamic profile is to release the drug in a controlled manner and site specific manner.

Microparticles are a type of drug delivery systems where the particle size ranges from one micron (one thousandth of a mm) to few mm. This microencapsulation technology allows protection of drug from the environment, stabilization of sensitive drug substances, elimination of incompatibilities, or masking of unpleasant taste. Hence, they play an important role as drug delivery systems aiming at improved bioavailability of conventional drugs and minimizing side effects[1].

Microparticulate drug delivery system is one of the processes to provide the sustained & controlled delivery of drug to long periods of time. They are small particles of solids or small droplets of liquids surrounded by walls of natural & synthetic polymer films of varying thickness & degree of permeability acting as a release rate controlling substance & have a diameter upto the range of 0.1µm-200µm[2] .

**Advantages of Microparticles**:

Recently, controlled release has become a very useful tool in pharmaceutical area, offering a wide range of actual and perceived advantages to the chronic diseases such as rheumatoid arthritis, osteoarthritis, and musculoskeletal disorders including degenerative joint conditions still demand long-term therapy. With the advent of microparticles following advantages were noted in the dosage forms

(1) Effective delivery of agents which re insoluble or sparingly soluble in water.

(2) They give the products which exhibit immediate release properties & can give 80% or more of active agent in about 10 minutes or less. Ex. Nimesulide.

(3) The technique provides the way for improving taste of an active agent.

(4) They increased the relative bioavailability of drugs.

(5) The formulation of microparticles also provides the method of targeting the drug delivery to specific sites.

(6) The microparticles hold great potential in reducing the dosage frequency & toxicity of various drugs.

(7) Microparticles in the form of microcapsules can also be used as carrier for drugs & vaccines as diagnostic agents & in surgical procedures.

(8) They can also be used to produce amorphous drugs with desirable physical properties.

(9) They also caused the reduction of the local side effects ex. GI irritation etc of drugs on oral ingestion.

(10) They provide the sustained release formulation with lower dose of drug to maintain plasma concentration & improved patient compliance.

(11) The PH triggered microparticles are used in immunization, transfection & gene therapy.

(12) Parentral microparticles have the advantage of administering high concentration of water

soluble drugs without severe osmotic effects at the site of administration.

(13) They also have an advantage of being stored in dry particle or suspension form with little or no loss of activity over an extended storage period.

(14) They are useful in administration of effervescent dosage form of medicaments to individual unable to chew. Ex. Debilitated patients having difficulty in swallowing solids & the elderly.

(15) In contrast, smaller microparticles need to be prepared for application to other sites such as the eye, lung, and joints [2].

**Disadvantages**

Although the advantages of microparticles are impressive there are certain limitations. These include:

1. The costs of the materials and processing of the controlled release preparation, which may be substantially higher than those of standard formulations.
2. The fate of polymer matrix and its effect on the environment.
3. The fate of polymer additives such as plasticizers, stabilizers, antioxidants and fillers.
4. Reproducibility is less.

5. Process conditions like change in temperature, pH, solvent addition, and evaporation/agitation may influence the stability of core particles to been capsulated.
6. The environmental impact of the degradation products of the polymer matrix produced in

hydrolysis, oxidation, solar radiation or biological response to heat, agents and the cost, time probability of success in securing government registration of the product, if required[3].

**Polymer & other substances used in microparticle preparation**

**Wall Materials**

The coating material can be selected from a wide variety of natural and synthetic polymers depending on the core material to be encapsulated and the desired characteristics. The amount of coating material used ranges from 3% to 30% of the total weight, which corresponds to a dry film thickness of less than 1–200 μm, depending on the surface to be coated[4].

**1. Natural or synthetic hydrophilic colloids**

These are large molecules that are soluble or dispersible in aqueous solutions. Here the capsule wall presents a good barrier to oily and hydrophobic materials, but it is usually a poor barrier to hydrophilic substances. Hydrophobic colloids are realized in encapsulating water-soluble drugs. Soluble starch & its derivatives including Amylodextrin, Amylopectin & Carboxy methyl starch is used as wall forming material in solid microsphere preparation. Some examples of natural and synthetic hydrophilic colloids are agar acrylic, polymers, polyacrylic acid, poly acryl methacrylate, gelatin, poly(lactic acid), pectin(poly glycolic acid), waxes (poly hydroxyl butyrate-co-valerate), cellulose derivatives, cellulose acetate phthalate, Nitrate, Ethyl cellulose, Hydroxy ethyl cellulose, Hydroxypropylcellulose, Hydroxy propyl methyl cellulose, Hydroxypropylmethylcellulose phthalate, Methyl cellulose, Sodium carboxymethylcellulose, Poly(orthoesters), Polyurethanes, Poly(ethylene glycol), Poly(ethylene vinyl acetate), Polydimethylsiloxane, Poly(vinyl acetate phthalate), Polyvinyl alcohol, Polyvinyl pyrrollidone, shellac[5].

**2. Biocompatible polymer**

It includes poly (lactic) acid (PLA), poly (glycolic acid) (PLGA). PLGA is a water-insoluble polymer; strength, hydrophobicity, and pliability are the significant physical advantages. As a polymeric vehicle, biocompability, biodegradability,

predictability of degradation, ease of fabrication, and regulatory approval are features that make PLGA desirable for medical applications. Natural polymers Albumin Chitin Starch, Collagen Chitosan Dextrin, Gelatin, Hyaluronic acid, Dextran, Fibrinogen, Alginic acid ,Casein, Fibrin, Poly(ortho esters). Polyalkylcyanoacrylate, Polyanhydrides.

The bioavailability enhancers used are lysophatide, lysophosphatidyl choline. Permeability modifier & membrane fluidity modifier used include enamines like phenyl alanine enamine. Malonates like diethylene ox methylene malonate, salicylates, bile salts, fusidates etc[5].

**3. Biodegradable polymer**

Advances in polymer science have opened up possibilities for using a wide variety of polymeric materials as drug delivery systems. Biodegradable polymers, by virtue of their ability to degrade in the body naturally, offer enormous advantages over conventional drug delivery systems. It eliminated the need for surgery and also does not elicit any adverse reactions from the body. Polymeric drug delivery systems are mainly intended to deliver the drug over a period of time. Some of the materials that are currently being used/studied for controlled drug delivery include poly (methyl methacrylate), poly (vinyl alcohol), polyacrylamide, polyethylene glycol, polylactic acid, polyglycolic acid, polylacticglycolic acid, and polyanhydrides . Most biodegradable polymers are designed to degrade as a result of hydrolysis of the polymer chains into biologically acceptable and progressively smaller compounds. For example in the case of polylactic glycolic acid, the polymer would eventually break down into lactic and glycolic acid, enter the Krebs cycle and further broken down into carbon dioxide and water. Drugs formulated in polymeric devices are released either by diffusion through the polymeric barrier, or by erosion of the polymer material, or by a combination of both diffusion and erosion mechanisms. A wide variety of natural and synthetic biodegradable polymers have been investigated for drugtargeting or prolonged drug release. Amongst them, the thermoplastic aliphatic poly (esters) like PLA, PGA, and especially PLGA have generated tremendous interest due to their excellent biocompatibility and biodegradability. The wide acceptance of the lactide/glycolide polymers as suture materials, made them an attractive candidate for biomedical applications like ligament reconstruction, tracheal replacement, surgicaldressings, vascular grafts, nerve, dental, and fracture repair. The

first work on parenteral controlled release of drugs using PLA was reported by Wise and Beck. Since then various polymeric devices like microspheres, microcapsules, nanoparticles, pellets, implants and films have been fabricated using these polymers for the delivery of a varietyof drug classes. Also they are easy to formulate into drug carrying devices for variety of applications, such as, orthopedic drug delivery; they have been approved by the FDA for drug delivery use[6].

**Techniques of microparticle preparation:**

When preparing controlled release microspheres, the choice of the optimal method has utmost importance for the efficient entrapment of the active substance. Various pharmaceutically acceptable techniques for the preparation of microparticles have been given. Some of the methods include:

1. Emulsion–solvent evaporation (o/w, w/o, w/o/w).
2. Phase separation (non solvent addition and solvent partitioning).
3. Interfacial polymerization.
4. Spray drying.
5. Emulsion extraction process.
6. Jet milling technique.
7. Fluidization & solvent precipitation method.

**1. Emulsion-solvent evaporation**

The solvent evaporation method involves the emulsification of an organic solvent (usually methylene chloride) containing dissolved polymer and dissolved/dispersed drug in an excess amount of aqueous continuous phase, with the aid of an agitator. The concentration of the emulsifier present in the aqueous phase affects the particle size and shape. When the desired emulsion droplet size is formed, the stirring rate is reduced and evaporation of the organic solvent is realized under atmospheric or reduced pressure at an appropriate temperature. Subsequent evaporation of the dispersed phase solvent yields solid polymeric microparticles entrapping the drug.

The solid microparticles are recovered from the suspension by filtration, centrifugation, or lyophilization solvent such as acetonitrile, are emulsified into an immiscible lipophilic phase, with light mineral oil commonly being used, in the presence of an oil-soluble surfactant such as Span. However, an important drawback of using an oil external phase is cleaning up the final product. The oil has to be removed using organic solvents such as n-hexane. Diphenyl hydramine

hydrochloride, mitomycin C, adriamycin, cephradine and cefadroxil[6]. Phenobarbitone and timolol maleate are some examples of drugs that have been encapsulated by this procedure.

The concentration of the emulsifier present in the aqueous phase affects the particle size and shape. When the desired emulsion droplet size is formed, the stirring rate is reduced and evaporation of the organic solvent is realized under atmospheric or reduced pressure at an appropriate temperature. Subsequent evaporation of the dispersed phase solvent yields solid Polymeric microparticles entrapping the drug. The solid microparticles are recovered from the suspension filtration, centrifugation, or lyophilization[6].

**Single-Emulsion Solvent Evaporation**:

**i. O/W Emulsion Solvent Evaporation Technique**

For emulsion solvent evaporation, there are basically two systems from which to choose: oil-in-water (o/w) or water-in-oil (w/o). Oil-in- water emulsion was to encapsulate progesterone. Afterward lipid-soluble drugs such as steroids, local anesthetics, bleomycin sulfate, doxorubicin, chlorpromazine, naltrexone and promethazine were encapsulated successfully.

In general, solvent evaporation method is particularly suitable for the microencapsulation of lipophilic drugs that can be either dispersed or dissolved in the dispersed phase of a volatile solvent. Sansdrap and Moes suggested that in order to obtain batches of microspheres with reproducible sizes, manufacturing factors such as emulsifier concentration, stirring rate and organic phase volume should be under control[7].

**ii. Oil-in-Oil Emulsification–Solvent Evaporation Technique**

Oil-in-oil (sometimes referred as water-in oil) emulsification process was developed for the encapsulation of highly water soluble drugs[7]. In this technique, polymer and drug, contained in a polar solvent such as acetonitrile, are emulsified into an solvent such as acetonitrile, are emulsified into an immiscible lipophilic phase, with light mineral oil commonly being used, in the presence of an oil-soluble surfactant such as Span. However, an important drawback of using an oil external phase is cleaning up the final product.

The oil has to be removed using organic solvents such as n-hexane. Diphenyl hydramine hydrochloride, mitomycinC, adriamycin, cephradine and cefadroxil,

phenobarbitone and timolol maleate are some examples of drugs that have been encapsulated by this procedure.

**iii. Multiple-Emulsion Technique (w/o/w)**

Multiple-emulsion or double-emulsion technique is appropriate for the efficient incorporation of water soluble peptides, proteins, and other macromolecules. This method allows the encapsulation of water-soluble drugs with an external aqueous phase when compared to nonaqueous methods as the o/o solvent evaporation or organic phase separation. In brief, the polymers are dissolved in an organic solvent and emulsified into an aqueous drug solution to form a w/o emulsion. This primary emulsion is re-emulsified into an aqueous solution containing an emulsifier to produce multiple w/o/w dispersion.

The organic phase acts as a barrier between the two aqueous compartments, preventing the diffusion of the active material toward the external aqueous phase. Microspheres manufactured by the (w/o/w method exhibit various morphologies such as porous or nonporous external polymer shell layers enclosing hollow, macro porous, or micro porous internal structures, depending on different parameters.

**2. Phase Separation/Coacervation:**

The term coacervation was suggested for the first time by two Dutch scientists. The word coacervation comes from the latinacervus, meaning aggregation, and the prefix co, signifying the preceding union of the colloidal particles. In this process, both the drug and the polymer should be insoluble in water, while a water-immiscible solvent is required for the polymer.

Coacervation/phase separation can be obtained by temperature change, nonsolvent or salt addition, incompatible polymer addition, and polymer–polymer interaction. Drugs belonging to different pharmacological groups have been encapsulated. Antibiotics, Anti-inflammatory agents, analgesics, and antihypertensive are some of these groups[7].

**Description of Coacervation/Phase Separation Methods:**

This method is divided into two main groups: aqueous and organic. Aqueous phase separation has been subdivided by Bungenberg de Jong and Kruyt as complex and simple coacervation.

**i. Simple coacervation**

Simple coacervation can be accomplished by the addition of chemical compounds with a high affinity for water, such as salts and alcohols. In principle,

simple coacervation can be brought about in any aqueous polymer solution when temperature, pH, solvent, and salt are properly chosen.

This process depends primarily on the degree of hydration produced. The added substances cause two phases to be formed, one rich in colloid droplets and the other poor. Its principal requirement is the creation of an insufficiency of water in a part of the total system. Figure illustrates the preparation of microcapsules by simple Coacervation.

The microencapsulation process can be explained by the following steps.

a. Dispersion of the core material in an aqueous solution of the polymer.
b. Creation of insufficiency of water for the hydrophilic colloid and the deposition of the coacervate around the core.
c. Gelation of the coacervate and hardening of the microcapsules.

**ii. Complex coacervation**

This technique of complex coacervation was first described by Phares and Sperandio. It involves neutralization of the charges on the colloids and depends primarily on pH. This is accomplished by mixing two colloids of opposite charges together. The encapsulation process in complex coacervation consists of four steps:

a. Preparation of a hydrophilic colloid solution.
b. Addition of a second hydrophilic colloid solution of opposite charge to induce coacervation.
c. Deposition around the core.
d. Gelation of the coacervate and hardening of the microcapsules [7].

**Organic Phase Separation Methods**

Organic phase separation is the inverse of the aqueous phase separation process in that the wall-containing phase is hydrophobic in nature and the core material is water miscible. The principle is to enclose water-soluble material with a polymeric wall material in an organic solvent by adding a nonsolvent or a second polymeric material to induce phase separation[8].

**3. Interfacial Polymerization Method**

Interfacial polymerization technique is one in which two monomers, one oil-soluble and the other water-soluble, are employed and a polymer is formed on the droplet surface. The method involve the reaction of mono meric units located at the

interface existing between a core material substance & a continuous phase in which the core material is dispersed[8].

**4. Spray drying**

Spray drying is used to protect sensitive substances from oxidation based on the atomization of a solution by compressed air and drying across a current of warm air. Microparticle formulation by spray drying is conducted by dispersing a core material in a coating solution, in which the coating substance is dissolved & in which the core material is insoluble, & then by atomizing the mixture into an air stream. The heated air causes removal of solvent from the coating solution thus causing formation of the microcapsule[8].

**5, Emulsion extraction process**

This method which is used to prepare microparticles, involves organic phase removal by extraction of the organic solvent. The method involves external phase miscible organic solvent such as mixing acetonitril and dichloromethane; organic phase is removed by extraction with liquid paraffin. The rate of solvent removal by extraction method depends on the temperature of external phase, ratio of emulsion volume to the mineral oil and the solubility profile of the polymer Several methods and techniques are potentially useful for the preparation of Microparticles in the field of controlled drug delivery. The type and the size of the microparticles, the entrapment, release characteristics and stability of drug in microparticles in the formulations are dependent on the method used. One of the most common methods of preparing microparticles is the single emulsion technique. Poorly soluble, lipophilic drugs are successfully retained within the microparticles prepared by this method. However, the encapsulation of highly water soluble compounds including protein and peptides presents formidable challenges to the researchers. The successful encapsulation of such compounds requires high drug loading in the microparticles, prevention of protein and peptide degradation by the encapsulation method involved and predictable release, both rate and extent, of the drug compound from the microparticles. The above mentioned problems can be overcome by using the double emulsion technique, alternatively called as multiple emulsion technique. Aiming to achieve this various techniques have been examined to prepare stable formulations utilizing w/o/w, s/o/w, w/o/o, and s/o/o type double emulsion methods. This article reviews the current state of the art in double emulsion based technologies for the

preparation of microparticles including the investigation of various classes of substances that are pharmaceutically and biopharmaceutically active[8].

**6, Jet milling technique**

An innovative technique for solvent free preparation of microparticles is described. Microparticles were prepared by a melt grinding technique which consists of three consecutive steps of melting in case of placebo microparticles or co-melting of polymer and drug in case of drug loaded microparticles, respectively, and pregrinding. In a final jet milling step the reduction of the particle size and smoothening of the microparticle surface occurred. Different polymers of PLA and PLGA type were utilised. The influence of the preparation parameters during the process were investigated according to microparticle properties like particle size distribution, habitus or surface morphology by executing a 2 factorial design. The minimum mean particle size distribution ($x_{50}$ value) reached 4–6 μm. Scanning electron microscopy revealed that non-porous microparticles with a smooth surface were prepared. The release pattern of estrioltriacetate loaded microparticles of Resomer® R 202H nearly followed a zero order release kinetic over a period of 21 days without an initial burst effect. The preparation process can be carried out in a reproducible manner. The results demonstrate that microparticle preparation is possible by the following unique melt grinding technique without using organic solvents[9].

**7. Fluidization & solvent precipitation method**

There are three commonly used fluid-bed process: top, tangential and bottom spray methods. When the granules are coated by the top-spray granulator system, granules usually have a porous surface and an interstitialvoid space, therefore, the bulk density of produced granules is usually lower than that attainable by granulation techniques. A rotating-disk method (tangential-spray coating method), which combinescentrifugal, high-density mixing and the efficiency of fluid bed drying, yields a product that has higher bulk density but still has some interstitial void space. It yields particle that are less friable and more spherical in shape. the solid core particle are fluidized by air pressure and a solution of wall materials is sprayed on to the particles from the bottom of the fluidization chamber parallel to the air stream. At the spraying nozzle is immersed in the airflow and sprays the coating materialsconcurrently into the fluidized particles, the coating solution droplets travel

only a short distance before contacting the solid particles. As a result the film is applied more evenly and the coated film is more homogenous. The coated particles are lifted on the air stream, which dries the coating as the particles are carried away from the nozzle. The particlesrise on the air stream, the settle down, and then begin another cycle. The cycles until the desired film thickness are achieved. It is well suited for uniform coating of particle with a polymeric membrane in a single operation[9].

**Gelatin Dispersion**

This is a specific embodiment of a more general approach in which the polymer filaments or monomer subunits used in forming the microparticles are mixed with a suspension of proteins, such as agar, gelatin, or albumin. One method employs alginate plus $Ca^{+2}$ in producing the particles. The mixture is then dispersed under conditions effective to produce desired sized particles containing the mixture components. In the case of gelatin containing particles, the mixture may be cooled during the dispersion process to produce gelled particles having a desired size. The particles are then treated under polymerization and/or cross linking conditions, preferably under conditions that do not also lead to cross linking of gelatin molecules to the polymer structure. After microparticle formation, the gelatin molecules may be removed from the structure, with such in a decondensed form, e.g., by heating the material or enzymatic digestion[8].

**Superficial antisolvent precipitation technique**

This technique is useful if the drug is insoluble in gas & gas is soluble in liquid. The drug is dissolved in polymeric solution of suitable solvent. Then the application of an antisolvent decreases the solubility of material the dissolved in solution leading to microparticle beads formation.

**pH-triggered microparticle** - Microparticles that are designed to release their payload when exposed to acidic conditions are provided as a vehicle for drug delivery[9]. Any therapeutic, diagnostic or prophylactic agent may be encapsulated in a lipid-protein-sugar or polymer matrix with a pH- triggering agent to form microparticles. Preferably the diameter of the pH triggered microparticles ranges from 50 nm to 10 μm. The matrix of the particles may be prepared using any known lipid (e.g., DPPC), protein (e.g., albumin), or sugar (e.g., lactose).

The matrix of the particles may also be prepared using any synthetic polymers such as polyesters. The process of formulation include providing an agent & contacting with a pH triggering agent & component selected from lipid, proteins, sugars & spray drying the resultant mixture to create microparticles. Typically, the pH triggering agent is a chemical compound including polymers with a pKa less than 7. pH triggering agent used is poly (butyl methacrylateco-(2-dimethyl amino ethyl) methacrylate-co-methyl methacrylate) (1:2:1) i.e. Eudragit 110.

The pH triggered microparticles release the encapsulated agent when exposed to an acidic environment such as in phagosome or endosome of a cell that has taken up particles thereby allowing for efficient delivery of agent intracellularly[9].

**Condensed phase microparticles**

They are an alternative method for storing & administration of drugs at high concentration in condensed phase with sizes ranging between 0.05-50 microns. They consists of

1. Matrix of cross linked polyionic polymer filaments capable of swelling from a condensed phase to an expanded, decondensed phase or state, when the matrix is exposed to monovalent counter ions.
2. Small molecules entrapped in microparticle matrix, with such in its condensed phase.
3. Polyvalent counter ions effective to retard the release of small molecules from the micro particles, when exposed to monovalent counter ions.

The composition is useful in delivery of vehicle for reagents is unstable on storage, or where it is desirable to introduce reagent at a selected step in reaction The method of preparation include infusing the compound into polymer suspended in a decondensed phase typically containing 10-200 millimole concentration of monovalent counter ions leading to hydration & increase in size. After compound infusion into open particle matrices, multivalent counter ion mainly Calcium ion is added to fully condense the microparticle. This technique is used for small, water soluble drug molecules. They are having advantages that high concentration of water soluble rugs can be administered without severe osmotic effect at site of administration thus they are essentially nonosmotic until they decompose & release drug[10].

**Evaluation of Microparticles**

The various evaluation techniques for microparticle preparation are as follows:

**1. Particle shape & size determination**

It can be done by microscopy, sieve analysis, laser light scattering, coulter counter method, photon correlation spectroscopy.

a. Crystallinity can be evaluated by differential scanning calorimetery analysis.
b. Shape & surface morphology can be studied by freeze fracture microscopy & freezes etch. electron microscopy.
c. Laser diffractometer & light microscope is also used to measure the size range of the microparticles.
d. Size analysis of all the batches of prepared microparticles can be carried out using a set of standard sieves ranging from 10-100 meshes. The microparticles

are passed through the set of sieves and the amount retained on each sieve is weighed. The arithmetic average diameter is determined by dividing the total weight size by 100[9-10].

**2. Bulk and Tap density**

Aerated bulk density is when the volume of the particle is at a maximum, caused by aeration, just prior to complete breakup of the bulk. The amount to which the particles collapse and fill voids between the particles depends on some powder properties, including particle shape, interparticle friction, and cohesion.

**Tapped density**

Tapped bulk density is, the maximum bulk density that can be achieved without deformation of the particles. In practice, it is generally unrealistic to attain this theoretical tapped bulk density, and a lower value obtained after tapping the particle in a standard manner is used[11-12].

**3. The Thermal Properties**

The Thermal Properties are detected by Differential Scanning Calorimetry (DSC), Thermo gravimetric analysis.

**Differential Scanning Calorimetry (DSC)**

In DSC the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature. Both the sample and reference are maintained at nearly the same temperature throughout the experiment. Generally, the temperature program for a DSC analysis is designed such that the sample holder temperature increases linearly as a function of time. The reference sample should have a well-defined heat capacity over the range of temperatures to be scanned.

**Thermogravimetric analysis or thermal gravimetric analysis (TGA)**

It is a type of testing that is performed on samples to determine changes in weight in relation to change in temperature. Analysis is carried out by raising the temperature gradually and plotting weight (percentage) against temperature. The temperature in many testing methods routinely reaches 1000°C or greater, but the oven is so greatly insulated that an operator would not be aware of any change in temperature even if standing directly in front of the device. After the data are obtained, curve smoothing and other operations may be done such as to find the exact points of inflection[11].

**4. Electrostatic interaction** is detected by rheological & FTIR assays (Fourier Transform Infra red spectroscopy) using potassium bromide pellets.

**5. Peptide entrapment & entrapment efficacy** can be evaluated by HPLC.

**6. The Drug release studies** was evaluated by USP method II or dissolution test method using phosphate buffer PH 6.8 with the temperature of release medium at 37°C ±0.5 & then assaying spectophotometrically[11-12].

**7. Release kinetics** to model the dissolution profile from the microparticles system two different mathematical differential equation can be used i.e., (1) first order equation; (2) Higuchi's square root of time equation.

1, First order model can be expressed as

$$Mt / M = 1- e- k_1 t$$

2, Higuchi's square root of time model is given by

$$Mt / M = k_H t^{1/2}$$

Where $M_t$ is the amount of drug released at time t, M is the maximal amount of drug released at infinite time, $k_1$ and $k_H$ are the rate constants for first order and Higuchi model, respectively.

Stability studies were evaluated to find out stable product under storage. Micro particles can be stored in Blass bottles at elevated temperature i.e. 4±1ºC freezing temperature, 25±1ºC room temperature, & 50±1ºC hot temperature for a period of 30 days & observed for change in drug content & morphology[12-13].

**Applications of microparticles**

1. Application areas of microcapsules include pharmaceutical and biotechnology products, cosmetics, diagnostic aids, biological filtration devices, veterinary and zoo technical products, foods and food additives, flavors, fragrances, detergents, paints, agricultural chemicals, adhesives, industrial chemicals, household products, packaging, textiles, photographic and graphic arts materials.
2. These microcapsules are important in providing sustained and controlled release, improving drug stability, reducing vaporization of volatile oils, protecting moisture/light/oxidation–sensitive drugs, masking unpleasant taste and odor, converting liquids to powders, and separating incompatible substances within a single system.
3. Amoxicillin, ampicillin, bacampicillin, cephalexin, cephradine, chloramphenicol, clarithromycin, eryithromycin, potassium pheneticillin, ofloxacin, and ciprofloxacin are some examples of the encapsulated antibiotics.

4. Anti-inflammatory drugs are another group in which microencapsulation is employed. Diclofenac sodium, flufenamic acid, glaphenine, hydrocortisone, ibuprofen, indomethacin, naproxen, oxyphenbutasone, and prednisone are examples of encapsulated drugs in this group.
5. Sulfadiazine, sulfamethizole, sulfamethoxazole, sulfamerazine, and sulfisoxazole are some representatives of sulfa drugs that are encapsulated.
6. Furosemide, chlorothiazide, and sulfonamide were encapsulated in order to prepare sustained release formulations that would offer the advantage of avoiding short periods of peak diuresis observed with the conventional formulations.
7. Isosorbide-5-mononitrate (IS-5-MN), dihydralazine sulfate, piretanide and propranolol HCl, captopril, nicardipin, and dipyridamole are examples of microencapsulated antihypertensives. IS-5-MN microcapsules were optimized and formulate to sustain the action and to overcome the tolerance developed in conventional preparations.
8. Vitamins A, B1, B2, B6, B12, C, D, were encapsulated to provide formation of smooth- and thick-walled microcapsules largely prevented the aggregation of microcapsules and showed low dissolution rate.
9. Converting Liquids to Free-Flowing Powders Citrus essential oil, cod liver oil, benzaldehyde, carbon tetrachloride, and oil droplets were coated and recovered as fine powders. The authors have stated that the bulk droplet size of the encapsulated material appeared to be a factor in the strong capsule wall, which protects against vaporization and oxidation.
10. Air filled micro particles are used in echocardiography & other ultrasonic imaging techniques. They are also used as opacifier or reflectivity enhancers in cosmetics[12].
11. Solid microspheres are of particulars used in nasal delivery of drugs including polypeptides, insulin, somatostain, metolopromide etc.
12. PH triggered micro particles have been used to deliver drugs by various means ex-by IV inject, intra dermal injection, rectally, orally, intra vaginally, inhalationally, mursoual delivery etc.

13. They are also used for administering. An antigenic epitote of a pathogen or a tumor.
14. The micro particles are useful in transficting cells & gene therapy.
15. Condensed phase micro particles are used as stable strong kit for enzymes, antibodies, dye[13].

# *Literature Review*

# LITERATURE REVIEW

**Nagendra R et al**[13]., prepared and evaluated microparticles containing Oxybutynin Chloride for controlled release using HPMC and Ethyl cellulose by solvent evaporation method using different ratios of drug to polymer and prepared microparticles were characterized. The method is simple, rapid and economical and does not imply the use of toxic organic solvents. The method used was suitable for both water-soluble and insoluble drugs. The Drug to polymer blend ratio of 2.5:10:5 (F5) produced discrete spherical microparticles. The DSC thermogram obtained for the pure drug and formulation shows no significant shift in the endothermic peaks confirming the stability of the drug in the formulation. From the results of drug content determination, it can be inferred that there was a proper and uniform distribution of drug in the micro particles. The in vitro drug release data showed the release of a drug in a controlled manner.

**SHAYEDA et al**[14]., studied on formulation and evaluation of floating microparticles of metoprolol succinate. They concluded floating multiunit drug delivery system was successfully prepared by non-aqueous emulsion solvent evaporation method for a freely water soluble drug, Metoprolol succinate by using Eudragit RLPO and RSPO, Eudragit S 100. The formulation containing drug to Eudragit RSPO in 1:6 ratio could sustain the release for 8 hours. However, further pharmacokinetic studies are needed to understand and confirm the effective absorption of Metoprolol succinate from floating microparticles. Further, the microspheres can also be compressed into tablets, filled into capsules, or formulated into oral suspension for reconstitution.

**Mitra Jelvehgar et al**[15]**;** done formulation and evaluation of in-vitro characterization of gastic-mucoadhesive microparticles/discs containing antidiabetic drug like metformin hydrochloride (MH) for oral administration with a view of combination of two polymers Carbomer 934p (cp) and Ethylcellulose (EC). They concluded that in their study % entrapment efficiency was higher for F2microparticles than other formulations. While practical yield obtained was higher for (1:3) F2 microparticles. The particle size analysis revealed that all formulations gave particles in the range of 794.33-1071. 52µm which is suitable for oral administration of formulation. Increase in the mucoadhesive polymer led to increase in mucoadhesion and degree of swelling. However, Carbopol showed higher mucoadhesion and swelling degree than

EC. As the concentration of mucoadhesive polymer increased, the drug releases also decreased proportionally, because it produced hydrogel on the mucus. From all the parameters studied, it can be concluded that microparticles and discs do not have significant difference together ($p > 0.05$). MH microparticles are better gastric-adhesive delivery system for the formulation of MH for gastro-intestinal administration. Thus, the formulated microspheres seem to be a potential candidate as oral controlled drug delivery system for diabetes therapy.

**KPR Chowdary et al[16]., ** Prepared and Evaluated of Gliclazide Micro particulate Drug Delivery Systems Employing Starch Acetateper. They conculded Gliclazide release from the starch acetate micro particles was slow and spread over longer periods of time. The drug release depended on the proportion of core: coat in the micro particles. A good linear relationship ($R^2$=0.826) between percent coat and release rate (ko)was observed. The relationship could be expressed by the linear equation, y=12.18-0.173x where x is percentcoat and y is release rate (ko). Gliclazide release from the starch acetate micro particles was by non fickian (anomalous) diffusion. Formulation F2 prepared using a Core: coat ratio of 8:2 gave slow, controlled and complete release (100%) of Gliclazide over 12hours. As such formulation F2 is considered as a promising micro particulate DDS for oral control release of Gliclazide over 12 hours for b.i.d administration.

**Josephine Lenojenita et al[17]., ** performed on formulation and evaluation of microparticles containing curcumin for colorectal cancer. The micro particles prepared were filled in hard gelatin capsules which were enteric coated with cellulose acetate phthalate (CAP) which prevent the burst effect of capsule in acidic pH of stomach. The in vitro release study showed that there was no drug release in pH 1.2 acidic buffer, drug release was between 66.12% - 71.87% for 10 hrs in phosphate buffer and controlled by Fickian diffusion mechanism. They concluded from their investigation that Eudragit microspheres are promising controlled release carriers for colon-targeted delivery of curcumin.

**Dv Gowda et al[18]., ** prepared and evaluated ranolazine (RNZ) microparticle with mixture of eudragit (EU) and ethylcellulose (EC) by the phase separation method for controlled release. Spherical discrete microparticles were obtained and it exhibited good micromeritic properties. From the results of the particle size analysis it was

clear that all the process variables were within the limits and the process is reproducible. FT-IR studies indicated that there was no interaction between the drug and the polymers in the prepared microparticles. The DSC thermograms obtained from the pure drug and the formulation showed no significant shift in the endothermic peaks confirming the stability of the drug in the formulation. The in vitro drug release studies showed that the release of the drug was found to be diffusion-controlled. Results of dissolution studies for formulation (3:1) F5 and the marketed product showed that both have nearly similar release profiles. From their results, they concluded that the microparticles formulation is easy to administer, simple and economical with increased patient compliance. Hence RNZ could be formulated into microparticles as a controlled drug release dosage form by the phase separation method. These results demonstrate the potential use of EU and EC combinations for fabrication of delivery systems of other water soluble drugs in a controlled manner.

**Pai Rohan V et al[19]., ** developed and evaluated chitosan microparticles based dry powder inhalation formulations of rifampicin and rifabutin. They conculded Both rifampicin and rifabutin-loaded microparticles had MMAD close to 5 μm and FPF values of 21.46% and 29.97%, respectively. In vitro release study in simulated lung fluid pH 7.4 showed sustained release for 12 hours for rifampicin microparticles and up to 96 hours for rifabutin microparticles, the release being dependent on both swelling of the polymer and solubility of the drugs in the dissolution medium. In vitro uptake studies in U937 human macrophage cell line suggested that microparticles were internalized within the macrophages. In vivo acute toxicity study of the microparticles in Sprague Dawley rats revealed no significant evidence for local adverse effects. Both rifampicin and rifabutin-loaded microparticles had MMAD close to 5 μm and FPF values of 21.46% and 29.97%, respectively. In vitro release study in simulated lung fluid pH 7.4 showed sustained release for 12 hours for rifampicin microparticles and up to 96 hours for rifabutin microparticles, the release being dependent on both swelling of the polymer and solubility of the drugs in the dissolution medium. In vitro uptake studies in U937 human macrophage cell line suggested that microparticles were internalized within the macrophages. In vivo acute toxicity study of the microparticles in Sprague Dawley rats revealed no significant evidence for local adverse effects. Thus, spray-dried microparticles of the

anti-tubercular drugs, rifampicin and rifabutin, could prove to be an improved, targeted, and efficient system for treatment of tuberculosis.

**BALAGANI. PAVAN KUMAR et al[20].,** formulated and evaluated glimepiride loaded cellulose acetate microparticles by using an emulsion solvent evaporation technique.They coculded The resulting microparticles obtained by solvent evaporation method were free flowing in nature. The mean particle size of microparticlesranges from 145.26 – 187.42 µm and encapsulation efficiency ranges from 90.38 – 96.00%. The infrared spectra and differential scanningcalorimetry thermographs confirmed the stable character of Glimepiride in the drug-loaded microparticles. Scanning electron microscopy revealedthat the microparticles were spherical in nature. In vitro release studies revealed that the drug release was sustained up to 12 hrs. The releasekinetics of Glimepiride from optimized formulation followed zero-order and peppas mechanism. The mechanism of drug release from the microparticles was found to be non-Fickian type. Which will not only sustain the release of drug but also manage complicacy of the diabetes in a better manner.

**ZhiYong Qian et al[21].**, prepared alginate coated chitosan microparticles for vaccine delivery. They concluded that their prepared alginate coated chitosan microparticles, with mean diameter of about 1µm, was suitable for oral mucosal vaccine. Moreover, alginate coating onto the surface of chitosan microparticles could modulate the release behavior of (Bovine Serum Albumin) BSA from alginate coated chitosan microparticles and could effectively protect model protein (BSA) from degradation in acidic medium in vitro for at least 2 h. In all, the prepared alginate coated chitosan microparticles might be an effective vehicle for oral administration of antigens.

**Maja Simonoska Crcarevska et al[22].**, Done a chitosan coated ca–alginate microparticles loaded with budesonide for delivery to the inflamed colonic mucosa. They concluded Using a novel one-step spray-drying process uncoated and Eudragit S 100 coated chitosan–Ca–alginate microparticles efficiently loaded with budesonide (BDS), with bioadhesive and controlled release properties in GIT, were prepared. Microparticles were spherical with mean particle size of 4.05–5.36 µm, narrow unimodal distribution and positive surface charge. A greater extent of calcium chloride limited the swelling ratio of beads, while swelling behaviour of

coated beads was mainly determined by properties of enteric coating. Comparing the release profiles of formulations, under different pH conditions, influence of polymer properties and concentration of cross-linker on the rate and extent of drug release was evident. Coating has successfully sustained release of BDS in buffers at pH 2.0 and 6.8, while providing potential for efficient release of BDS at pH 7.4. Release data kinetics indicated influence of erosion and biodegradation of polymer matrix on drug release from microparticles. Prepared formulations were stable for 12 months period at controlled ambient conditions. that coated microparticles prepared by one-step spray-drying procedure could be suitable candidates for oral delivery of BDS with controlled release properties for local treatment of inflammatory bowel diseases.

**Nahla S Barakat and Alanood S Almurshedi et al[23].,** designed and developed gliclazide loaded chitosan microparticles for oral sustained drug delivery: invitro/invivo evaluation. They concluded Gliclazide release from the starch acetate micro particles was slow and spread over longer periods of time. The drug release depended on the proportion of core: coat in the micro particles. A good linear relationship ($R^2$=0.826) between percent coat and release rate (ko)was observed. The relationship could be expressed by the linear equation, y=12.18-0.173x where x is percentcoat and y is release rate (ko). Gliclazide release from the starch acetate micro particles was by non fickian (anomalous) diffusion. Formulation F2 prepared using a Core: coat ratio of 8:2 gave slow, controlled and complete release (100%) of Gliclazide over 12hours. As such formulation F2 is considered as a promising micro particulate DDS for oral control release of Gliclazide over 12 hours for b.i.d administration. from their results, they suggests that gliclazide–chitosan microparticles are a valuable system for the sustained delivery of gliclazide.

**Ahmed S. Zidan. et al[24].,** Formulated anastrozole microparticles as biodegradable anticancer drug carriers. They concluded The purpose of this study was to develop poly(d,1-lactic-coglycolic acid) (PLGA)-based anastrozole microparticles for treatment of breast cancer. An emulsion/extraction method was used to prepare anastrozole sustained-release PLGA-based biodegradable microspheres. Gas chromatography with mass spectroscopy detection was used for the quantitation of the drug throughout the studies. Microparticles were formulated and characterized in terms of encapsulation efficiency, particle size distribution, surface morphology, and drug release profile. Preparative variables such as concentrations of stabilizer, drug-

polymer ratio polymer viscosity, stirring rate, and ratio of internal to external phases were found to be important factors for the preparation of anastrozole-loaded PLGA microparticles. Fourier transform infrared with attenuated total reflectance (FTIR-ATR) analysis and differential scanning calorimetry (DSC) were employed to determine any interactions between drug and polymer. An attempt was made to fit the data to various dissolution kinetics models for multiparticulate systems, including the zero order, first order, square root of time kinetics, and biphasic models. The FTIR-ATR studies revealed no chemical interaction between the drug and the polymer. DSC results indicated that the anastrozole trapped in the microspheres existed in an amorphous or disordered-crystalline status in the polymer matrix. The highest correlation coefficients were obtained for the Higuchi model, suggesting a diffusion mechanism for the drug release from their results, anastrozole microparticles with PLGA could be an alternative delivery method for the long-term treatment of breast cancer.

**Hombreiro Perez M, et al[25]., d**one a preparation and evaluation of poly(ϵ-caprolactone) microparticles containing both a lipophilic and a hydrophilic drug. They concluded that In vitro release studies revealed a controlled release of nifedipine and propranolol HCl from microparticles prepared by the o/w-method; a burst release of propranolol HCl was observed from microparticles prepared by the w/o/w-method. In conclusion, microparticles containing both a hydrophilic and a Lipophilic drug were successfully prepared.

**Sakchai Wittaya-areekul et al[26].,** studied on preparation and in vitro evaluation of mucoadhesive properties of alginate/chitosan microparticles containing prednisolone. They conclued that, prednisolone release at a pH of 6.8 after 4 h was between 63 and 79% for the particles prepared by the one-step method and between 57 and 88% for the particles prepared by the two-step method with a prednisolone drug load of 5 and 10% (w/v), respectively. Their results showed that depending on the preparation method these chitosan coated alginate particles show different mucoadhesiveness whereas their other properties are not statistically significant different.

**Manmohan Singh et al[27].,** prepared a cationic microparticles: A potent delivery system for DNA vaccines. They concluded that, the microparticles released intact and functional DNA over 2 weeks *in vitro*. In addition, the microparticles induced higher levels of marker gene expression *in vivo*. After intra muscular immunization,

the microparticles induced significantly enhanced serum antibody responses in comparison to naked DNA. Moreover, the level of antibodies induced by the microparticles was significantly enhanced by the addition of a vaccine adjuvant, aluminum phosphate. In addition, in contrast to naked DNA, the cationic microparticles induced potent cytotoxic T lymphocyte responses at a low dose.

**Philip F.Builders et al[28].,** studied on preparation and evaluation of mucinated sodium alginate microparticles for oral delivery of insulin. The microparticles formed were generally multi-particulate, discrete and free flowing. The prepared microparticles were filled into hard gelatin capsules and the *in vitro* insulin release as well as the blood glucose reduction after oral administration to diabetic rabbits were determined. Before insulin loading, microparticles were round and smooth, becoming fluffier, less spherical and larger with rough and pitted surface after insulin loading. The insulin content of the microparticles increased with increase in their sodium alginate content. The various insulin-loaded microparticles prepared with the mucinated sodium alginate when encapsulated exhibited lag time before insulin release. The time taken to reach maximum insulin release from the various formulations varied with the mucin–sodium alginate ratio mix. The mean dissolution time of insulin from the microparticles prepared with sodium alginate, mucin, sodium alginate: mucin ratios of 1:1, 3:1 and 1:3 was $11.21 \pm 0.75$, $3.3 \pm 0.42$, $6.69 \pm 023$, $8.52 \pm 0.95$ and $3.48 \pm 0.65$ (min.), respectively. The percentage blood glucose reduction for the subcutaneously administered insulin was significantly ($p < 0.001$) higher than for the formulations. The blood glucose reduction effect produced by the orally administered insulin-loaded microparticles prepared with three parts of sodium alginate and one part of mucin after 5 h was, however, equal to that produced by the subcutaneously administered insulin solution, an indication that it is an effective alternative for the delivery of insulin.

**Jianhua Zheng et al[29].,** perfomed on preparation and evaluation of floating-bioadhesive microparticles containing clarithromycin for the eradication of Helicobacter pylori. They concluded that in vitro buoyancy and drug-release testing confirmed the good floating and sustained-release properties of (chitosan- alginate-ethylcellulose ) CAEMs. About 74% of the CAEMs floated in an acetate buffer solution for 8 h, and 90% of the clarithromycin contained in the CAEMs was released within 8 h in a sustained manner. In vivo mucoadhesive testing showed that

61% of the CAEMs could be retained in the stomach for 4 h. Under a pretreatment with omeprazole, the clarithromycin concentration in gastric mucosa of the CAEM group was higher than that of the clarithromycin solution group. These results suggest that CAEMs might be a promising drug delivery system for the treatment of H. pylori infection.

**Mvan der Lubben et al[30].,** performed on Chitosan microparticles for oral vaccination: preparation, characterization and preliminary in vivo uptake studies in murine Peyer's patches. They concluded that these results were verified using field emission scanning electron microscopy, which demonstrated the porous structure of the chitosan microparticles, thus facilitating the entrapment of ovalbumin in the microparticles. Loading studies of the chitosan microparticles with the model compound ovalbumin resulted in loading capacities of about 40%. Subsequent release studies showed only a very low release of ovalbumin within 4 h and most of the ovalbumin (about 90%) remained entrapped in the microparticles. Because the prepared chitosan microparticles are biodegradable, this entrapped ovalbumin will be released after intracellular digestion in the Peyer's patches. Initial in vivo studies demonstrated that fluorescently labeled chitosan microparticles can be taken up by the epithelium of the murine Peyer's patches. Since uptake by Peyer's patches is an essential step in oral vaccination, these results show that the presently developed porous chitosan microparticles are a very promising vaccine delivery system.

**Mcuna M.J and Alonso D Torres et al[31].,** performed on Preparation and in vivo evaluation of mucoadhesive microparticles containing amoxycillin–resin complexes for drug delivery to the gastric mucosa. They conculded, microparticles consisting of amoxycillin-loaded ion-exchange resin encapsulated in mucoadhesive polymers (polycarbophil and Carbopol 934) were prepared with the aim of increasing the efficacy of amoxycillin in the treatment of peptic ulcers by achieving targeted delivery to the gastric mucosa and prolonged drug release. An oil-in-oil solvent evaporation technique was conveniently modified in order to obtain polymer microparticles containing multiple amoxycillin–resin cores. Polycarbophil microparticles were spherical, Carbopol 934 microparticles irregular. In vitro release of amoxycillin was rapid with or without a polymer coating. Gastrointestinal transit in rats was investigated by fluorescence microscopy using particles loaded with

fluorescein instead of amoxycillin: gastric residence time was longer, and the distribution of the particles on the mucosa apparently better, without any polymer coating.

**J.AKo et al[32].,** prepared a chitosan microparticles with tripolyphosphate (TPP) by ionic crosslinkingand done a characterization intended for controlled drug delivery. They concluded **t**he particle sizes of TPP-chitosan microparticles were in range from 500 to 710 μm and encapsulation efficiencies of drug were more than 90%. The morphologies of TPP-chitosan microparticles were examined with scanning electron microscopy. As pH of TPP solution decreased and molecular weight (MW) of chitosan increased, microparticles had more spherical shape and smooth surface. Release behaviors of felodipine as a model drug were affected by various preparation processes. Chitosan microparticles prepared with lower pH or higher concentration of TPP solution resulted in slower felodipine release from microparticles. With decreasing MW and concentration of chitosan solution, release behavior was increased. The release of drug from TPP-chitosan microparticles decreased when cross-linking time increased. These results indicate that TPP-chitosan microparticles may become a potential delivery system to control the release of drug.

**R.P.Raffin et al[33].,** perfomed on Preparation, characterization, and in vivo anti-ulcer evaluation of pantoprazole-loaded microparticles.They conculded Pantoprazole is an important drug in the treatment of acid-related disorders. This work concerns the preparation and characterization of gastro-resistant pantoprazole-loaded microparticles prepared using an O/O emulsification/solvent evaporation technique. The in vivo activity of the pantoprazole-loaded Eudragit S100 microparticles was carried out in rats. Furthermore, tablets containing the microparticles were also investigated. Microparticles presented spherical and smooth morphologies (SEM) and they remained intact in the inner surface of tablets. DSC and IR analyses showed that pantoprazole was physically and molecularly dispersed in the polymer. In vivo anti-ulcer evaluation showed that the microparticles were able to protect rat stomachs against ulcer formation, while the drug aqueous solution did not present activity. Drug dissolution profiles from tablets demonstrated slower release than untabletted microparticles. Weibull equation was the best model for describing the drug release

profiles from microparticles and tablets. As regards the acid protection, tablets showed a satisfactory drug protection in acid medium (61.05 ± 8.09% after 30 min).

**Herbert Hoyeret al[34].**, performed on Preparation and evaluation of microparticles from thiolated polymers via air jet milling. They conculded Microparticles from (thiolated) chitosan had a positive charge whereas microparticles from (thiolated) poly(acrylic acid) were negatively charged. The maximum protein load for microparticles based on chitosan, chitosan–glutathione (Ch–GSH), poly(acrylic acid) (PAA) and for poly(acrylic acid)–glutathione (PAA–GSH) was 7 ± 1%, 11 ± 2%, 4 ± 0.2% and 7 ± 2%, respectively. The release profile of all microparticles followed a first order release kinetic. Chitosan (0.5 mg), Ch–GSH, PAA and PAA–GSH particles showed a 31.4-, 13.8-, 54.2- and a 42.2-fold increase in weight, respectively. No significant cytotoxicity could be found. Thiolated microparticles prepared by jet milling technique were shown to be stable and to have controlled drug release characteristics. After further optimizations the preparation method described here might be a useful tool for the production of protein loaded drug delivery systems.

**NahedHegazy et al[35].,** peformed on Preparation and in vitro evaluation of pyridostigmine bromide microparticles.They conculded Pyridostigmine bromide (PB) is an anticholinesterase agent whose aqueous solubility is high and which has a short elimination half-life. Its dosage rate in the treatment of myastenia gravis is frequent due to the short half-life and it exhibits side effects. Microparticles designed to deliver a pharmaceutical active ingredient efficiently at the minimum dose and also to enhance stability, reduce side effects and modify drug release were prepared in this study using an acrylic polymer (Eudragit) as the vehicle by the spray-drying technique. The drug was either dissolved or dispersed in the polymeric solution and following the preparation of microparticles using different ratios of ingredients, characterization studies including the determination of shape, particle size distribution, amount loaded, release and stability of PB were performed. The results obtained were compared to those of pure PB. Drug release from microparticles could be modified and was found to depend on the shapes of the microparticles. In vitro evaluation results indicate that the frequent dosage and side effects of pure PB may be reduced with the formulation of microparticles[39].

**Behzad Sharif Makhmal Zadeh et al[36].**, performed on preparation and evaluation of sodium diclofenac loaded chitosan controlled release microparticles using factorial design.They conculed The sodium diclofenac loaded chitosan microparticles were prepared by ionic cross-linking with sodium tripolyphosphate successfully. Loading efficiency of sustained release microparticles to be touched by percentage of cross-linking agent, Tween 80 and drug-chitosan proportion. Chitosan microparticles provided suitable sustained release pattern in the manner that with increasing in percentage of Tween 80, the released amount reduced.

**J. Ravi Kumar Reddy et al[37].,** performed on formulation and evaluation of microparticles of metronidazole. They concluded the results of their study that delayed release microparticles of Metronidazole could be prepared by emulsion solvent evaporation technique by using the polymers like CAP, HPMCP, EL- 100 and ES-100. The microparticles prepared using CAP, HPMCP, EL-100 and ES-100 showed little amount of brittleness. This defect can be rectified by adding the plasticizer like castor oil, tween and span. The correct percentage of plasticizer to be added can be determined. Comparative drug release study revealed that the formulated product (microparticles) have more sustained effect than the marketed product.

**G.C.Bazzo et al[38].,**performed on Poly(3-hydroxybutyrate)/chitosan/ketoprofen or piroxicam composite microparticles: Preparation and controlled drug release evaluation.They conculed Pyridostigmine bromide (PB) is an anticholinesterase agent whose aqueous solubility is high and which has a short elimination half-life. Its dosage rate in the treatment of myastenia gravis is frequent due to the short half-life and it exhibits side effects. Microparticles designed to deliver a pharmaceutical active ingredient efficiently at the minimum dose and also to enhance stability, reduce side effects and modify drug release were prepared in this study using an acrylic polymer (Eudragit) as the vehicle by the spray-drying technique. The drug was either dissolved or dispersed in the polymeric solution and following the preparation of microparticles using different ratios of ingredients, characterization studies including the determination of shape, particle size distribution, amount loaded, release and stability of PB were performed. The results obtained were compared to those of pure PB. Drug release from microparticles could be modified and was found to depend on the shapes of the microparticles. In vitro evaluation

results indicate that the frequent dosage and side effects of pure PB may be reduced with the formulation of microparticles.

**NahedHegazy et al[39].,** peformed on Preparation and in vitro evaluation of pyridostigmine bromide microparticles.They conculded Pyridostigmine bromide (PB) is an anticholinesterase agent whose aqueous solubility is high and which has a short elimination half-life. Its dosage rate in the treatment of myastenia gravis is frequent due to the short half-life and it exhibits side effects. Microparticles designed to deliver a pharmaceutical active ingredient efficiently at the minimum dose and also to enhance stability, reduce side effects and modify drug release were prepared in this study using an acrylic polymer (Eudragit) as the vehicle by the spray-drying technique. The drug was either dissolved or dispersed in the polymeric solution and following the preparation of microparticles using different ratios of ingredients, characterization studies including the determination of shape, particle size distribution, amount loaded, release and stability of PB were performed. The results obtained were compared to those of pure PB. Drug release from microparticles could be modified and was found to depend on the shapes of the microparticles. In vitro evaluation results indicate that the frequent dosage and side effects of pure PB may be reduced with the formulation of microparticles.

**KC Ofokansi et al[40].,** performed on Formulation and Evaluation of Glutaraldehyde-Crosslinked Chitosan Microparticles for the Delivery of Ibuprofen.They conculded Entrapment of ibuprofen in glutaraldehyde-cross-linked chitosan microparticles can beexploited to target and control the release of the drug and possibly reduce its gastro-erosive sideeffects.Entrapment of ibuprofen in glutaraldehyde-cross-linked chitosan microparticles can beexploited to target and control the release of the drug and possibly reduce its gastro-erosive side effects.

**Rania O. Salama et al[41] .,** performed on Preparation and Evaluation of Controlled Release Microparticles for Respiratory Protein Therapy. They conculded All formulations had relatively good aerosolisation performance when compared to conventional dry powder inhalation (DPI) formulations although increasing PVA percentage had a negative effect on the aerosol performance in vitro. Analysis of the difference and similarity factors for the release profiles indicated significant differences with respect to PVA concentration. Furthermore, cell toxicity analysis

indicated PVA to have limited effect on cell viability after 24 h exposure. A series of protein-based inhalation formulations have been developed and tested, and shown to be suitable for controlled release in the respiratory tract.

**Priscileila Colerato Ferrar et al[42].,** performed on Floating ability and drug release evaluation of gastroretentive microparticles system containing metronidazole obtained by spray drying.They conculded Floating microparticles were successfully obtained and characterized to potential gastroretentive effect, and possible use for gastric disease treatment, such as H. pylori infections. Floating microparticles composed by chitosan and one hydrophilic (HPMC) or one hydrophobic (EC) polymer were developed. Physicochemical characterization has shown the drug encapsulation and did not indicate chemical linkage occurrence between components. The microparticles floating ability study revealed no difference among the HPMC:CS samples, while microparticles containing a higher amount of EC presented poor flotation. The drug release study demonstrated better control of the MT release by EC:CS 1:3 sample, and similar profiles among HPMC:CS samples. However, the microparticles composed by HPMC and CS presented the best relationship between floating ability and controlled drug release and it is believed to be ideal for floating gastroretentive systems regarding safe administration.

# *Drug Profile*

## LIST OF MATERIALS USED AND MANUFACTURERS

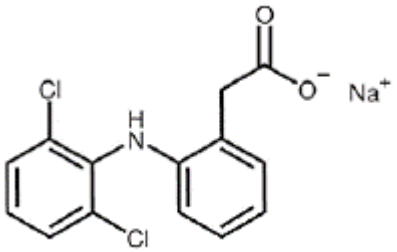
### List of Materials used and Manufactures

| S. No. | MATERIALS | MANUFACTURES |
|---|---|---|
| 1. | Diclofenac sodium | Aarti Drugs Limited, Chennai. |
| 2. | Sulfamethoxazole | Aarti Drugs Limited, Chennai. |
| 3. | Pottasium dihydogen ortho phosphate | Nice Chemicals Pvt., Ltd., Cochin, India. |
| 4. | Sodium hydroxide | S.D. Fine-Chem Ltd., Mumbai, India. |
| 5. | Coconut oil | Local Market |
| 6. | Span – 80 | Reachem Laboratory Chemical Private Limited, Chennai. |
| 7. | Chitosan | Yarrow Chem Products, Mumbai. |
| 8. | Propylene glycol | Reachem laboratory Chemicals Private Limited, Chennai. |
| 9. | Poly ethylene glycol – 6000 | Loba Chemie Private Limited, Mumbai, India. |
| 10. | N – Cyclohexane | Avantor Performance Materials India limited, Maharashtra, India. |
| 11. | Carbomer-940 | Merck specialities Pvt., Ltd., Mumbai, India |

**Drug Profile- Diclofenac sodium**

**Drug** : Diclofenac sodium

**Structural formula :**




**Molecular formula :** $C_{14}H_{10}Cl_2NNaO_2$.

**Molecular weight :** 318.129 g/mol.

**Chemical name :** Sodium{2-[(2,6-dichlorophenyl) amino] phenyl} acetate.

**Category :** Non steroidal anti inflammatory drug (NASID) with analgesic actions.

**Dose:** 25 mg, 50 mg, 75 mg, 100 mg and 150 mg.

**Description:** White or slightly yellowish crystalline powder.

**Solubility:** Soluble in water, pH 7.2, ethanol, methanol and acetone.

**Melting point :** 283° C - 285°C[43].

**MECHANISM OF ACTION**

The exact mechanism of action for diclofenac, like that of other NSAIDs, is unknown. They appear to have anti-inflammatory, anti-pyretic, and analgesic properties. These are thought to be mediated via inhibition of prostaglandins. This inhibition of prostaglandins is itself mediated via the inhibition of the cyclooxygenase (COX) enzyme. There are two forms of the COX enzyme. COX-1 is involved in 'housekeeping' activities, such as mediating normal platelet function, regulating renal blood flow and providing cytoprotection of the gastric mucosa. COX-2 is involved in the response to tissue damage and mediates inflammation and pain. The COX-2 inhibitors are more selective in their inhibition of COX-2 relative to COX-1. The COX-2 inhibitors have been associated with higher rates of cardiovascular adverse events and it is hypothesised that this effect is a result of relative COX-2/COX-1 inhibition. While diclofenac is a traditional NSAID, it does display a preferential inhibition of COX-2 compared to COX-1[44].

**Parmacokintics**

**Bioavailabilty:** Well absorbed undergoes first-pass metabolism; only 50-60% of a dose reaches systemic circulation as unchanged drug. Peak plasma concentration usually attained within about 1 hour.

**Onset:** Single 50 mg or 100 mg doses of diclofenac potassium provide pain relief within 30 minutes.

**Duration:** Pain relief lasts up to 8 hours following administration of single 50 mg or 100 mg doses of diclofenac sodium.

**Food:** Food delays time to reach peak plasma concentration but do not affect extent of absorption following administration as conventional Delayed-release or extended-release tablets.

**Distribution**

**Extent:** Following oral adminitration, concentration in synovial fluid may exceed those in plasma. Plasma protein binding: >99%.

**Metabolism:** Metabolised in the liver via hydroxylation and conjucation. Some metabolites may exhibit anti-inflammatory activity.

**Elimination:** Excreted in urine (65%) and in feces via biliary elimination (35%) as metabolites.

**Half-life:** Oral preparations: 1-2 hours. Diclofenac epolamin Transdermal system. approximately 12 hours[45].

**Drug interactions**

Drugs that can potentially interfere with diclofenac sodium include diuretics, other nonsteroidal anti-inflammatory drugs, and some herbal supplements. These interactions may increase the risk of kidney damage, reduce the activity of diuretics, or make ACE inhibitors less effective, among other things. If diclofenac sodium drug interactions are a concern, your healthcare provider may adjust your dosage or monitor you more closely[46].

**Adverse effects**

**Central nervous system:** Headache

**Gastro intestional Tract:** Stomach problems, including gas, bloatincramping, constipation, and diarrhea. Upset stomach and/or bleeding in your stomach, esophagus, or intestines.

**Skin :** Rash.

**Indications**

Mild-to-moderate pain. Moderate-to-severe pain alone or in combination with opioid analgesics. For relief of the signs and symptoms of osteoarthritis. For relief of the signs and symptoms of rheumatoid arthritis[47].

**Dosage and adminitration**

**Adult :** For the relief of signs and symptoms of osteoarthritis 35 mg orally 3 times a day. For the relief of signs and symptoms of primary dysmenorrheal 50 mg orally 3 times a daily. For the management of mild to moderate acute pain (oral, IV) and moderate to severe pain alone or in combination with opioid analgesics (IV) 150 mg per day[48].

**Usual Adult Dose for Migraine:** Diclofenac potassium for oral solution packets: 50 mg (1 packet) orally once Comments: This drug is not indicated for the prophylactic therapy of migraine or for use in cluster headaches; the safety and efficacy of a second dose has not been established.

Use: For acute treatment of migraine with or without aura.

**Usual Adult Dose for Dysmenorrhea:** Diclofenac potassium immediate-release tablets: 50 mg orally 3 times a day Comments: An initial dose of 100 mg orally followed by 50 mg oral doses may provide better relief for some patients; initiate treatment upon appearance of the first symptoms and continue for a few days.

Use: For the relief of signs and symptoms of primary dysmenorrhea.

**Usual Adult Dose for Osteoarthritis:** Diclofenac free acid capsules: 35 mg orally 3 times a day Diclofenac potassium immediate-release tablets: 50 mg orally 2 or 3 times a day Diclofenac sodium enteric-coated tablets: 50 mg orally 2 or 3 times a day or 75 mg orally 2 times a day Maximum dose: 150 mg daily Diclofenac sodium extended-release tablets: 100 mg orally once a day.

Use: For the relief of signs and symptoms of osteoarthritis[49].

**Contraindications**

Diclofenac sodium is contraindicated in the following patients: Known hypersensitivity (e.g., anaphylactic reactions and serious skin reactions) to diclofenac or any components of the drug product[50].

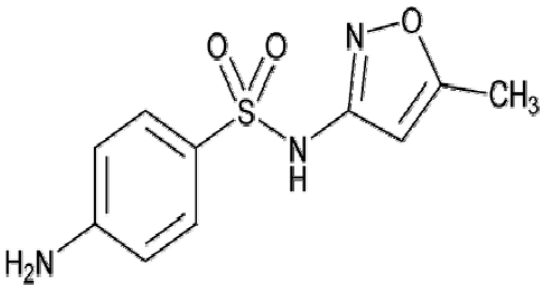
**Marketed products**

Diclogesic (Tab), Voltaren (Tab), Arthrotec (Tab), Arthrotec (injection), Reactin (Tab), Dylojet (Injection), Voveran-D (Tab), Amidol (Tab)[51].

**Drug Profile- Sulfamethoxazole**

**Drug** : Sulfamethoxazole

**Structural formula** :




**Molecular formula :** $C_{10}H_{11}N_3O_3S$

**Molecular weight :** 253.276 g/mol

**Chemical name :** 4-Amino-N-(5-methyl-1,2-oxazol-3-yl)benzenesulfonamide

**Category :** Sulfamethoxazole is a bacteriostatic antibacterial agent

**Dose :** 800 mg

**Description :** White and white crystalline powder

**Solubility :** Soluble in Ethanol, acetone, and methanol

**Melting point :** 169°C[52]

**Mechanism of action**

Sulfamethoxazole is a sulfonamide drug that inhibits bacterial synthesis of dihydrofolic acid by competing with para-aminobenzoic acid (PABA) for binding to dihydropteroate synthetase. Sulfamethoxazole is bacteriostatic in nature. Inhibition of dihydrofolic acid synthesis decreases the synthesis of bacterial nucleotides and DNA.

Sulfamethoxazole is normally adihydrofolate reductase inhibitor, which inhibits the reduction of dihydrofolic acid totetrahydrofolic acid. Studies have shown that bacterial resistance develops more slowly with the combination of the two drugs than with either Trimethoprim or Sulfamethoxazolealone.

Sulfamethoxazole is a sulfonamide bacteriostatic antibiotic that is Bactium. Sulfamethoxazole competitively inhibits Bactrim. Sulfamethoxazole Competitively inhibits dihydropteroate synthase preventing the formation of dihydropteroic acid, a precursor of folic acid which is required for bacterial growth[53].

**Parmacokinetics**

**Absorption:** Sulfamethoxazole is well-absorbed when administered topically. It is rapidly absorbed when it is orally administer.

**Distribution:** Sulfamethoxazole distributes into most body tissues as well as sputum, vaginal fluid, and middle ear fluid. It also crosses the placenta. About 70% of the drug is bound to plasma proteins. Its $T_{max}$ (or time to reach maximum drug concentration in plasma) occurs 1 to 4 hours after oral administration. The mean serum half-life of sulfamethoxazole is 10 hours. However, the half-life of the drug noticeably increases in people with creatinine clearance rates equal to or less than 30 ml/min. half-life of 22–50 hours has been reported for people with creatinine clearances of less than 10 ml/min.

**Metabolism:** Sulfamethoxazole is metabolized in the human liver to at least 5 metabolites. These metabolites are the N4-acetyl-, N4-hydroxy-, 5-methylhydroxy N4-acetyl-5-methylhydroxy-sulfamethoxazole metabolites, and an N-glucuronide conjugate. The CYP2C9 enzyme is responsible for the formation of the N4-hydroxy metabolite. In vitro studies suggest sulfamethoxazole is not a substrate of the P-glycoprotein transporter.

**Excretion:** Sulfamethoxazole is primarily renally excreted via glomerular filtration and tubular secretion.[ About 20% of the sulfamethoxazole in urine is the unchanged drug, about 15–20% is the N-glucuronide conjugate, and about 50–70 % is the acetylated metabolite. Sulfamethoxazole is also excreted in human milk[54].

**Drug interaction**

Caution with concomitant CYP2C8, 2C9, or OCT2 substrates. May potentiate oral anticoagulants (eg, warfarin), hypoglycemics, phenytoin, methotrexate, digoxin; monitor. May be potentiated by indomethacin. May increase risk of thrombocytopenia with diuretics (esp. thiazides). Nephrotoxicity with cyclosporine in renal transplant. Megaloblastic anemia with >25mg/week doses of pyrimethamine. May antagonize tricyclic antidepressants. May interfere with assays for serum methotrexate, creatinine[55].

**Adverse effects**

**Central nervous system:** Headache, dizziness.

**Gastro intestional tract:** Nausea, diarrhea, Abdominal or stomach pain

**Musculo skeletal system:** Lower pain back.

**Skin:** Itching, joint or muscle pain, blistering, peeling, or loosening of the skin, changes in skin color pale skin, rash, red skin lesions, often with a purple center.

**Indications**

Susceptible infections including UTIs (not for initial uncomplicated episodes), shigellosis, prophylaxis and treatment of Pneumocystis jiroveci pneumonia (PJP), travelers' diarrhea or acute exacerbations of chronic bronchitis in adults, acute otitis media in children[56].

**Dosage and administration**

**Adult:** 1 DS tab or 2 regular tabs every 12 hours for 5 days (shigellosis, travelers' diarrhea), or 10–14 days (UTIs), or 14 days (bronchitis). PJP treatment: 15–20mg/kg per day trimethoprim (75–100mg/kg per day sulfamethoxazole) in equally divided doses every 6 hours for 14–21 days; PJP prophylaxis: one DS tab daily. Renal impairment (CrCl 15–30mL/min): reduce dose by ½; CrCl <15mL/min: not recommended.

**Child:** <2 months: see Contraindications. ≥2 months: 8 mg/kg per day trimethoprim (40 mg/kg per day of sulfamethoxazole) in 2 divided doses at 12 hour intervals for 5 days (shigellosis) or 10 days (otitis media, UTIs). PJP treatment: as adult; PJP prophylaxis or renal impairment[57].

**Contraindications**

History of drug-induced immune thrombocytopenia with use of trimethoprim and/or sulfonamides. Megaloblastic anemia due to folate deficiency. Pediatrics <2 months. Marked hepatic damage. Severe renal insufficiency when renal status cannot be monitored[58].

**Marketed products**

Gantanol (Tab), Azo Gantanol (Tab), Bactrim (Tab), Bactrim DS (Tab) and Septra DS (Tab)[59].

# *Excipients Profile*

## POLY ETHYLENE GLYCOL

**Non proprietary names**

BP: Macrogols

JP: Macrogol 400

Macrogol 1500

Macrogol 4000

Macrogol 6000

Macrogol 20000

PhEur : Macrogols

USP-NF: Polyethylene Glycol

**Synonyms:**

Carbowax; Carbowax Sentry; Lipoxol; Lutrol E; macrogola; PEG; Pluriol E; polyoxyethylene glycol.

**Structural formula:**


**Chemical name:** A-Hydro-o-hydroxypoly(oxy-1,2-ethanediyl)

**Empirical formula:** $H(OCH_2CH_2)_nOH$

**Molecular weight:** 300 g/mol to 10,000,000 g/mol

**Melting point:** 55–63°C for PEG 6000

**Fuctional category:** Ointment base; plasticizer; solvent; suppository Base; tablet and capsule lubricant[60].

**DESCRIPTION:**

The USP32–NF27 describes polyethylene glycol as being an addition polymer of ethylene oxide and water. Polyethylene glycol grades 200–600 are liquids; grades 1000 and above are solids at ambient temperatures. Liquid grades (PEG 200–600) occur as clear, colorless or slightly yellow-colored, viscous liquids. They have a slight but characteristic odor and a bitter, slightly burning taste.PEG 600 can occur as a solid grades (PEG>1000) are white or off-white in color, and range in consistency from pastes to waxy flakes. They have a faint, sweet odor. Grades of PEG 6000 and above are available as free flowing milled powders.

**Solubility:** Polyethylene glycols are soluble in water Liquid polyethylene glycols are soluble in acetone, alcohols, benzene, glycerin, glycols. Solid polyethylene glycols are soluble in acetone, soluble in aliphatic hydrocarbons and ether but insoluble in fats, fixed oils, and mineral oil, alcohols, benzene, glycerin, and glycols. Solid polyethylene glycols are soluble in acetone dichloromethane, ethanol (95%), and methanol; they are slightly soluble in fats fixed oils, and mineral oil.

**Stability and storage conditions:** Polyethylene glycols are chemically stable in air and in solution, although grades with a molecular weight less than 2000 are hygroscopic. Polyethylene do not support microbial growth and do not become rancid. Polyethylene glycols and aqueous polyethylene glycol solutions can be sterilized by autoclaving, filtration, or gamma irradiation[61].

**Incompatibilities:** Chemical reactivity of polyethylene the glycols is mainly confined to the two terminal hydroxyl groups, which can be either esterified or etherified. However, all grades can exhibit all grades can exhibit some oxidizing activity owing to the presence of peroxide impurities and secondary products formed by autoxidation. Liquid and solid polyethylene glycol grades may be incompatible with some coloring agents. The antibacterial activity of certain antibiotics is reduce in polyethylene glycol bases particular that of penicillin and bacitracin. The preservative efficacy of the parabens[62].

## CHITOSAN

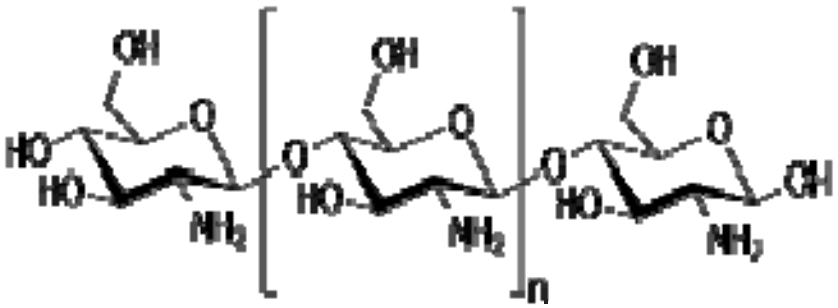
**Non proprietary names:**

BP: Chitosan Hydrochloride

PhEur: Chitosan Hydrochloride

**Synonyms:** 2-Amino-2-deoxy-(1,4)-b-D-glucopyranan; chitosan hydrochloridum; deacetylated chitin; deacetylchitin; b-1,4-poly-D-glucosamine;

**Structural formula:**




**Chemical name:** Poly-b-(1,4)-2-Amino-2-deoxy-D-Glucose.

**Empirical formula:** $(C_6H_{11}NO_4)_n$

**Molecular weight:** 10,000–10,00,000[63].

**Applications:**

Chitosan is used in cosmetics and is under investigation for use in a number of pharmaceutical formulations. The suitability and performance of chitosan as a component of pharmaceutical formulations for drug delivery applications has been investigated in numerous studies. These include controlled drug delivery applications use as a component of mucoadhesive forms, rapid release dosage forms, improved peptide delivery colonic drug delivery systems and use for gene delivery.

Chitosan has been processed into several pharmaceutical forms including gels films, beads, microspheres, tablets, and coatings for liposomes. Furthermore, chitosan may be processed into drug delivery systems using several techniques including spray-drying cocervation, direct compression, and conventional granulation processes.

**Fuctional category:** Coating agent; disintegrant; film- forming agent; mucoadhesive; tablet binder; viscosity increasing agent[64].

**Description:** Chitosan occurs as odorless, white or creamy-white powder or flakes. Fiber formation is quite precipitation and common during the chitosan may look 'cotton like'.

**Solubility:** Sparingly soluble in water; practically insoluble in ethanol (95%), other organic solvents, and neutral or alkali solutions at pH above approximately 6.5. Chitosan dissolves solvents, readily in dilute and concentrated solutions of most organic removed.

**Stability and storage conditions:** Chitosan powder is a stable material at room temperature, although it is hygroscopic after drying. Chitosan should be stored in a tightly closed container in a cool, dry place. The PhEur 6.5 specfies that chitosan should be stored at a temperature of 2–8°C.

**Incompatibilities:** Chitosan is incompatible with strong oxidizing agents[65].

## PROPYLENE GLYCOL

**Non proprietary names:**

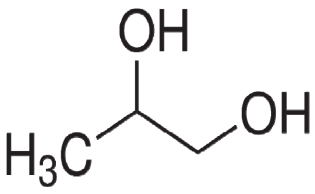
JP : Propylene glycol

PhEur : Propylene glycolum

USP : Propylene Glycol

**SYNONYMS:** 1,2-Dihydroxypropane; E1520; 2-Hydroxypropanol; methyl ethylene glycol; methyl glycol; propane-1,2-diol; propylene glycolum.

**Structural formula:**




**Chemical name:**

Propane-1,2- diol 1,2 –dihydroxypropane 1,2-popanediol methyl glycol

**Empirical formula:** $C_3H_8NO_2$

**Molecular weight:** 76. 10 g/cm$^3$

**Melting point:** - 59 ° C

**Solubility:** Soluble in water, Soluble in ethanol, soluble in diethyl ether, Soluble in acetone, Soluble in chloroform.

(Lowther A et al. Systemic contact dermatitis from propylene glycol.Dermatitis 2008; 19(2): 105–108.)

**Stability and Storage Conditions :** At cool temperatures, propylene glycol is stable in a well-closed container, but at high temperatures,in the open, it tends to oxidize, giving rise to products such as propionaldehyde, lactic acid, pyruvic acid, and acetic acid. Propylene glycol is chemically stable when mixed with ethanol (95%), glycerin, or water; aqueous solutions may be sterilized by autoclaving.

**Applications :** Propylene glycol is used as a solvent in many pharmaceuticals, including oral, injectable and topical formulations, such as for diazepam and lorazepam which are insoluble in water. Like ethylene glycol, propylene glycol is able to lower the freezing point of water, and so it is used as aircraft de-icing fluid.

**Fuctional category:** Food additives, Pharmaceutical aid treatment and prophylaxis of acetonemia in animals.

**Description:** Propylene glycol is a clear, colorless, viscous, practically odorless liquid, with a sweet, slightly acrid taste resembling that of glycerin.

**Incompatibilities:** The demixing behavior of 20 repsentatives of the system oligo propylene glycol is investigated at pressures up to 1500 bar. The degree of oligomerization range from 2 to 5 for the first component. The experimental results are compared with those for the previously studied system oligo propylene glycol/oligoisobutane. In both cases the observed upper critical temperatures increase with the number of monomeric units of the less polar component[66].

## COCONUT OIL

**Non proprietary names:**

JP : Coconut Oil

PhEur : Coconut Oil, Refined

USP-NF : Coconut Oil

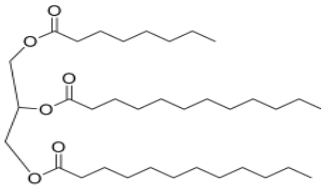
**Chemical name:**

EINECS 263-194-8**;** ((Coconut oil alkyl) amino) butyric acid, sodium salt; 2-Aminobutyric acid sodium salt; SCHEMBL15820003; Butanoic acid, 2-amino-, N-coco alkyl derivs, monosodium salts; Butanoic acid, 2-amino-, N-cocoalkyl derivs., monosodium salts.

**Synonyms:** Aceite de coco; cocois oleum raffinatum; coconut butter; copra oil; oleum coc Pureco 76; refined coconut oil.

**Empirical formula:** $C_4H_8NNaO_2$.

**Structural formula:**



**Molecular weight:** 640–680 g/mol

(CF Hung et al. The effect of oil components on the physico chemical properties and drug delivery of emulsions: tocol emulsion versus lipid emulsion. International Journal Pharmacy and research, 2007; 335(1–2): 193–202.)

**Melting point:** 76° C

**Applications:** Coconut oil has traditionally been used in ointments where it forms a readily absorbable base. It has been used particularly in preparations intended for application to the scalp, where it could be applied as a solid but would liquefy when applied to the skin. Coconut oil is readily saponified by strong alkalis even in the cold and, as the soap produced is not readily precipitated by sodium chloride, it has been used in the making of 'marine' soap. Coconut oil may be used in the formulation of a range of other preparations including emulsions and nanoemulsions, intranasal solutions and rectal capsules and suppositories. In addition, coconut oil has been reported to have antifungal activity against a range of Candida species[67].

**Fuctional category :**

Coconut oil is sometimes applied to the skin as a moisturizer, for neonatal health, and to treat eczema and a skin condition called psoriasis. Coconut oil is also used in hair products to prevent hair damage.

**Solubility:** Solubility in water: Coconut oil forms a white homogenous mixture

**Stability and Storage Conditions:** Coconut oil remains edible, and mild in taste and odor, for several years under ordinary storage conditions. However, on exposure to air, the oil readily oxidizes and becomes rancid, acquiring an unpleasant odor and strong acid taste. Store in a tight, well-filled container, protected from light at a temperature not exceeding 258C. Coconut oil may be combustible at high temperature, and may spontaneously heat and ignite if stored under hot and wet conditions.

**Description:** Coconut oil generally occurs as a white to light-yellow mass or colorless or light-yellow clear oil, with a slight odor characteristic of coconut and a mild taste. Refined coconut oil is a white or almost white unctuous mass.

**Incompatibilities:** Coconut oil reacts with oxidizing agents, acids and alkalis. Polyethylene is readily permeable to coconut oil[68].

**SPAN-80**

**Non proprietary names:** None adopted.

**Synonyms:** Afrodit; aluminum-saponite; auxite; cathkinite; ferroan saponite; griffithite; licianite; lucianite; piotine; zebedassite.

**Structural formula:** Saponite is composed of two tetrahedral layers formed by phylosilicate sheets and one octahedral layer. Common impurities include manganese, nickel, phosphorus, potassium, and titanium.

**Chemical name:**Saponite

**Empirical formula:** $(Ca0_5Na)_3(Mg,Fe_2þ)_3(Si,Al)_4O_{10}(OH)_{2\_4}H_2O$

**Solubility:** Saponite is composed of two tetrahedral layers formed by phylosilicate sheets and one octahedral layer. Common impurities include manganese, nickel, phosphorus, potassium, and titanium.

**Stability and Storage Conditions:**

Saponite is a stable material and should be stored in a cool, dry place.

**Applications:** Occurring clays such as magnesium Aluminum silicates and is therefore suitable for use in pharmaceutical formulation applications as an adsorbent viscosity-increasing agent, suspending agent, or as an oil-in- water emulsifying agent. Saponite, as a component magnesium aluminium silicates, is useful as a formulation component in semisolid cosmetic and health care products.

**Fuctional category:** Adsorbent; emulsifying agent; viscosity increasing agent.

**Description:** Saponite occurs as a white to off-white, dull powder composed of fine-grained. The material is greasy or soapy to the touch and swells on the addition of water.

**Incompatibilities:** May generate heat in contact with hydrofluoric acid[69].

## CARBOMER – 940

**Non proprietary names:**

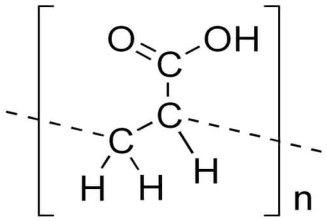
BP : Carbomers

PhEur : Carbomers

USP-NF : Carbomer

**Synonyms:** Acrypol; Acritamer; acrylic acid polymer; carbomera; Carbopol;carboxy polymethylene; polyacrylic acid; carboxyvinyl polymer; Pemulen; Tego Carbomer.

**Structural formula:**




**Chemical name:** Carbomer

**Empirical formula: (** $C_3H_4O_2)_n$

**Molecular weight:** 104 400 g/mol

**Melting point:** 260°C.

**Solubility:** Swellable in water and glycerin and, after neutralization, in ethanol (95%). Carbomers do not dissolve but merely swell to a remarkable extent, since they are three-dimensionally crosslinked microgels[70].

**Stability and Storage Conditions:**

Carbomers are stable, hygroscopic materials that may be heated at temperatures below 1048C for up to 2 hours without affecting their thickening efficiency. However, exposure to excessive temperatures can result in discoloration and reduced stability. Complete decomposition occurs with heating for 30 minutes at 2608C.

**Applications:** Carbomers are used in liquid or semisolid pharmaceutical formulations as rheology modifiers. Formulations include creams, gels, lotions and ointments for use in ophthalmic, rectal, topical and vaginal preparations.

**Functional category:** Bioadhesive material; controlled-release agent; emulsifying agent; emulsion stabilizer; rheology modifier; stabilizing agent; tablet binder.

**Description:** Carbomers are white-colored, 'fluffy', acidic, hygroscopic powders with a characteristic slight odor. A granular carbomer is also available (Carbopol 71G).

**Incompatibilities:** Carbomers are discolored by resorcinol and are incompatible with phenol, cationic polymers, strong acids, and high levels of electrolytes. Certain antimicrobial adjuvants should also be avoided or used at low levels. Trace levels of iron and other transition metals can catalytically degrade carbomer dispersions. Certain amino-functional actives form complexes with carbomer; often this can be prevented by adjusting the pH of the dispersion and/or the solubility parameter by using appropriate alcohols and polyols. Carbomers also form pH-dependent complexes with certain polymeric excipients. Adjustment of pH and/or solubility parameter can also work in this situation[71].

# *Aim and Plan of work*

## AIM AND PLAN OF WORK

The aim of this present work was to development of topical gels containing chitosan-and PEG-based microparticles loaded with dual drugs (diclofenac sodium and sulfamethoxazole) for bacterial skin infections.

The plan of this work can be outlined below,

- To carry out the preformulation studies for API
    - Description
    - Solubility
    - Melting point
- Drug excipients compatibility studies
    - Physical observation
- Formulation of microparticle contains diclofenac sodium and sulfamethoxazole.
- Evaluation of microparticle formulations
    - ❖ Percentage yield
    - ❖ Drug content
    - ❖ IR studies
    - ❖ Particle size determination by sieving method procedure
    - ❖ Dissolution studies
- Evaluation of microparticle formulations in gel
    - ❖ Gel preparation
    - ❖ Gel evaluation – Spreadability and consistency test
    - ❖ Microparticles mixed with gel
    - ❖ Permeation studies procedure
    - ❖ Release kinetics

# *Materials and Methods*

## LIST OF MATERIALS USED AND MANUFACTURERS

**List of Materials used and Manufactures**

| S. No. | MATERIALS | MANUFACTURES |
|---|---|---|
| 1 | Diclofenac sodium | Aarti Drugs Limited, Chennai. |
| 2 | Sulfamethoxazole | Aarti Drugs Limited, Chennai. |
| 3 | Pottasium dihydogen ortho phosphate | Nice Chemicals Pvt., Ltd., Cochin, India. |
| 4 | Sodium hydroxide | S.D. Fine-Chem Ltd., Mumbai, India. |
| 5 | Coconut oil | Local Market |
| 6 | Span – 80 | Reachem Laboratory Chemical Private Limited, Chennai. |
| 7 | Chitosan | Yarrow Chem Products, Mumbai. |
| 8 | Propylene glycol | Reachem laboratory Chemicals Private Limited, Chennai. |
| 9 | Poly ethylene glycol - 6000 | Loba Chemie Private Limited, Mumbai, India. |
| 10 | N – Cyclohexane | Avantor Performance Materials India limited, Maharashtra, India. |
| 11 | Carbomer-940 | Merck specialities Pvt., Ltd., Mumbai, India |

## INSTRUMENTS USED AND MANUFACTURERS

**List of Instruments used and Manufactures**

| S. No. | INSTRUMENTS | MANUFACTURES |
|---|---|---|
| 1 | Melting point Apparatus | Guna Enterprises, Chennai. |
| 2 | Mechanical Stirrer | Remi motors Limited. Mumbai, India. |
| 3 | pH – Meter | Toshniwal instruments Mfg. Pvt. Ltd, Ajmer, India |
| 4 | Water Bath | Guna Enterprises, Chennai. |
| 5 | U V –Spectrophotometer | SHIMADZU 8400S, Japan. |
| 6 | Dissolution Apparatus | Labindia analytical Instruments Limited, India. |
| **7** | Sieve sets | Jayant scientific IND, Mumbai. |

**Methodology**

**Preformulation studies**

Preformulation may be described as the process of optimizing a drug through determination of those physical and chemical properties considered important in the formulation of a stable, effective and safe dosage form. The possible interactions with the various components intended for use in the final drug product are also considered. It is an effort that encompasses the study of such parameters as dissolution, polymorphic forms and crystal size and shape, pH profile of stability and drug – excipients interactions, which may have a profound effect on a drug's physiological availability and physical and chemical stability. Preformulation involves the application of biopharmaceutical principles to the physicochemical parameters of drug substance are characterized with the goal of designing optimum drug delivery system.

**Scope**

Use of preformulation parameters maximizes the chances in formulating an acceptable, safe, efficacy and stable product. At the same time provides the basis for optimization of drug product quality.

Some of the important parameters evaluated during preformulation studies are following

- **Physicochemical evaluation of drug molecule**
    - Description
    - Solubility
    - Melting point
- **Compatibility studies of the drug with excipients**

**Description**

It is the initial evaluation during preformulation studies which assess the colour, odour, nature and taste of the substance. This was only a descriptive test[76-78].

**Solubility**

Aqueous solubility is an important physicochemical property of drug substance, which determines its systemic absorption and in turns its therapeutic efficacy.

**Table-1**

**Solubility Specifications**

| **Descriptive terms** | **Approximate volume of solvent in millilitres per gram of solute** |
|---|---|
| Very soluble | Less than 1 |
| Freely soluble | From 1 to 10 |
| Soluble | From 10 to 30 |
| Sparingly soluble | From 30 to 100 |
| Slightly soluble | From 100 to 1000 |
| Very slightly soluble | From 1000 to 10,000 |
| Practically insoluble | More than 10,000 |

**Melting point**

The temperature at which the first particle of the substance completely melts is regarded as melting point of the substance. The temperature at which the first particle start to melt and last particle completely melts is regarded as melting range[76]. Melting point determination was done for pure diclofenac sodium and sulfamethoxazole (separately and combine) by using melting point apparatus. Small amount of pure diclofenac sodium and sulfamethoxazole and combination of diclofenac sodium, sulfamethoxazole were taken in glass capillary tube whose one end was sealed by flame. The capillary tube containing samples was kept in the melting point apparatus and the melting point was noted.

**Physical drug-excipients compatibility studies**

In the some dosage form the drug is in intimate contact with one or more excipients, the latter could affect the stability of the drug. Knowledge of drug-excipients interactions therefore is very useful to the formulators in selecting appropriate excipients.

Compatibility studies were performed by preparing blend of different excipients with drug and stored at room temperature for one week. The initial state of mixture was noted and further evaluation for the possible occurrence of any interactions was checked after one week.

**Standard curve of diclofenac sodium and sulfamethoxazole**

**Preparation of 0.2 M potassium dihydrogen phosphate**

27.218 gm of potassium dihydrogen phosphate was dissolved in small quantity of distilled water and make upto 1000 ml with distilled water.

**Preparation of 0.2 M sodium hydroxide solution**

8 gm of sodium hydroxide was dissolved in little amount of distilled water and make upto 1000 ml with distilled water.

**Preparation of phosphate solution pH 7.4**

500 ml of 0.2 M Potassium dihydrogen phosphate solution and 391 ml of 0.2 M sodium hydroxide solution was mixed together and made upto 2000 ml with distilled water. Then it was adjusted to pH 7.4.

**Preparation of diclofenac sodium standard curve using phosphate buffer solution pH 7.4**

Accurately weighed 50 mg of diclofenac sodium and transferred to 50 ml standard measuring flask (SMF), small amount of pH 7.4 was added to dissolve diclofenac sodium, then the volume was make upto 50 ml with pH 7.4 to get a concentration of 1 mg/ml. From the above solution 20 ml was pipette out into a 200 ml volumetric flask and made upto the mark using phosphate buffer solution pH 7.4, to get a concentration of 100 µg/ml. It's a stock solution. From the stock solution, aliquots of 2 ml, 4 ml, 6 ml, 8 ml, 10 ml, 12 ml, 14 ml, 16 ml, 18 ml and 20 ml were pipetted out into a series of 100 ml volumetric flasks and made upto the 100 ml mark with phosphate buffer solution pH 7.4 to get drug concentration in the range of 2 to 20 µg/ml. The absorbance of the resulting solution was then measured at 276 nm in a UV double beam spectrophotometer against phosphate buffer solution pH 7.4 as blank. The standard curve was obtained by plotting concentration (µg/ml) values in X-axis and absorbance values in Y-axis.

**Table-2**

**Diclofenac sodium standard curve using phosphate buffer solution pH 7.4**

| S. No. | Concentration (µg/ml) | Absorbance at 276 nm | | | Average with SD* |
|---|---|---|---|---|---|
| | | 1st time | 2nd time | 3rd time | |
| 1 | 2 | 0.086 | 0.091 | 0.083 | 0.087±0.004 |
| 2 | 4 | 0.147 | 0.153 | 0.144 | 0.148±0.005 |
| 3 | 6 | 0.219 | 0.205 | 0.212 | 0.212±0.007 |
| 4 | 8 | 0.279 | 0.267 | 0.276 | 0.274±0.006 |
| 5 | 10 | 0.363 | 0.361 | 0.365 | 0.363±0.002 |
| 6 | 12 | 0.409 | 0.403 | 0.413 | 0.408±0.005 |
| 7 | 14 | 0.499 | 0.487 | 0.481 | 0.489±0.009 |
| 8 | 16 | 0.556 | 0.548 | 0.549 | 0.551±0.004 |
| 9 | 18 | 0.623 | 0.629 | 0.624 | 0.625±0.003 |
| 10 | 20 | 0.701 | 0.711 | 0.705 | 0.706±0.005 |

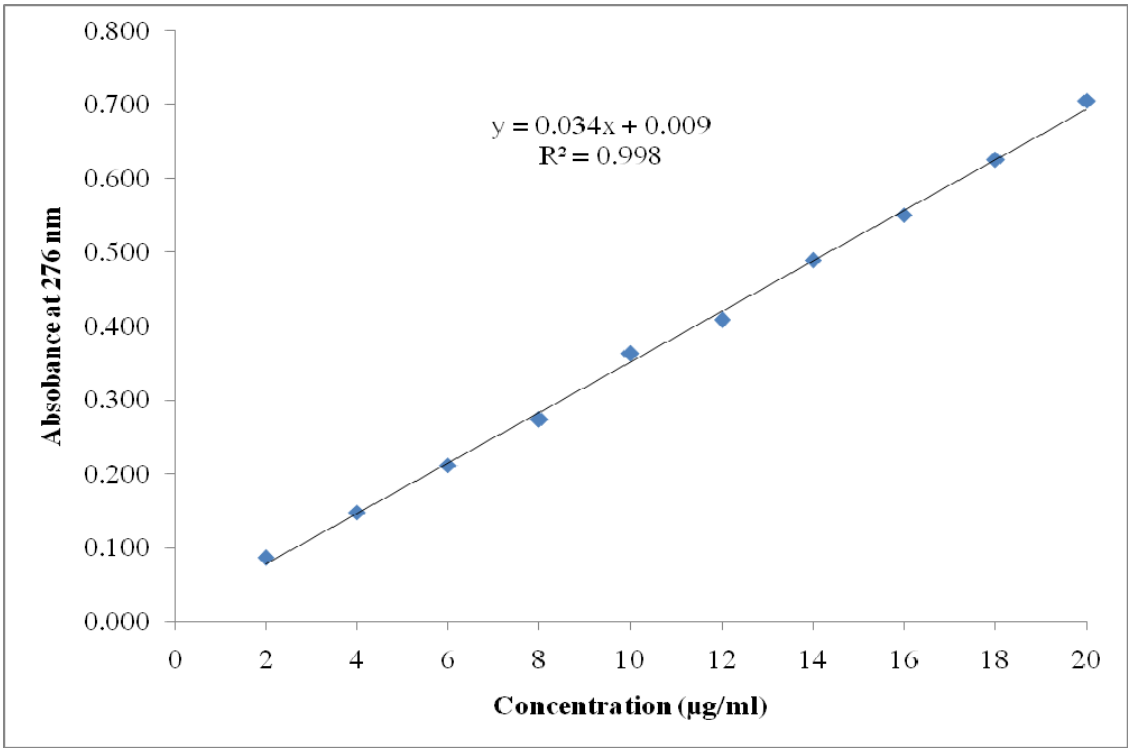



**Figure-1**

**Diclofenac sodium standard curve using phosphate buffer solution pH 7.4**

**Preparation of Sulfamethoxazole standard curve using phosphate buffer solution pH 7.4:**

Accurately weighed 100 mg of sulfamethoxazole and transferred to 100 ml SMF, small amount of pH 7.4 was added to dissolve diclofenac sodium, and then the volume was make upto 100 ml with pH 7.4 to get a concentration of 1 mg/ml. From the above solution 20 ml was pipette out into a 200 ml volumetric flask and made upto the mark using phosphate buffer solution pH 7.4, to get a concentration of 100 µg/ml. It's a stock solution. From the stock solution, aliquots of 2 ml, 4 ml, 6 ml, 8 ml, 10 ml, 12 ml, 14 ml, 16 ml, 18 ml and 20 ml were pipetted out into a series of 100 ml volumetric flasks and made upto the 100 ml mark with phosphate buffer solution pH 7.4 to get drug concentration in the range of 2 to 20 µg/ml. The absorbance of the resulting solution was then measured at 257 nm in a UV double beam spectrophotometer against phosphate buffer solution pH 7.4 as blank. The standard curve was obtained by plotting concentration (µg/ml) values in X-axis and absorbance values in Y-axis.

**Table-3**

**Sulfamethoxazole standard curve using phosphate buffer solution pH 7.4**

| S. No. | Concentration (µg/ml) | Absorbance at 257 nm | | | Average with SD* |
|---|---|---|---|---|---|
| | | 1st time | 2nd time | 3rd time | |
| 1 | 2 | 0.134 | 0.136 | 0.135 | 0.1350±0.0010 |
| 2 | 4 | 0.279 | 0.271 | 0.275 | 0.2750±0.0040 |
| 3 | 6 | 0.395 | 0.393 | 0.391 | 0.3930±0.0020 |
| 4 | 8 | 0.524 | 0.525 | 0.521 | 0.5233±0.0021 |
| 5 | 10 | 0.668 | 0.663 | 0.667 | 0.6660±0.0026 |
| 6 | 12 | 0.791 | 0.799 | 0.782 | 0.7907±0.0085 |
| 7 | 14 | 0.902 | 0.914 | 0.919 | 0.9117±0.0087 |
| 8 | 16 | 1.044 | 1.041 | 1.043 | 1.0427±0.0015 |
| 9 | 18 | 1.18 | 1.15 | 1.17 | 1.1667±0.0153 |
| 10 | 20 | 1.31 | 1.27 | 1.36 | 1.3133±0.0451 |

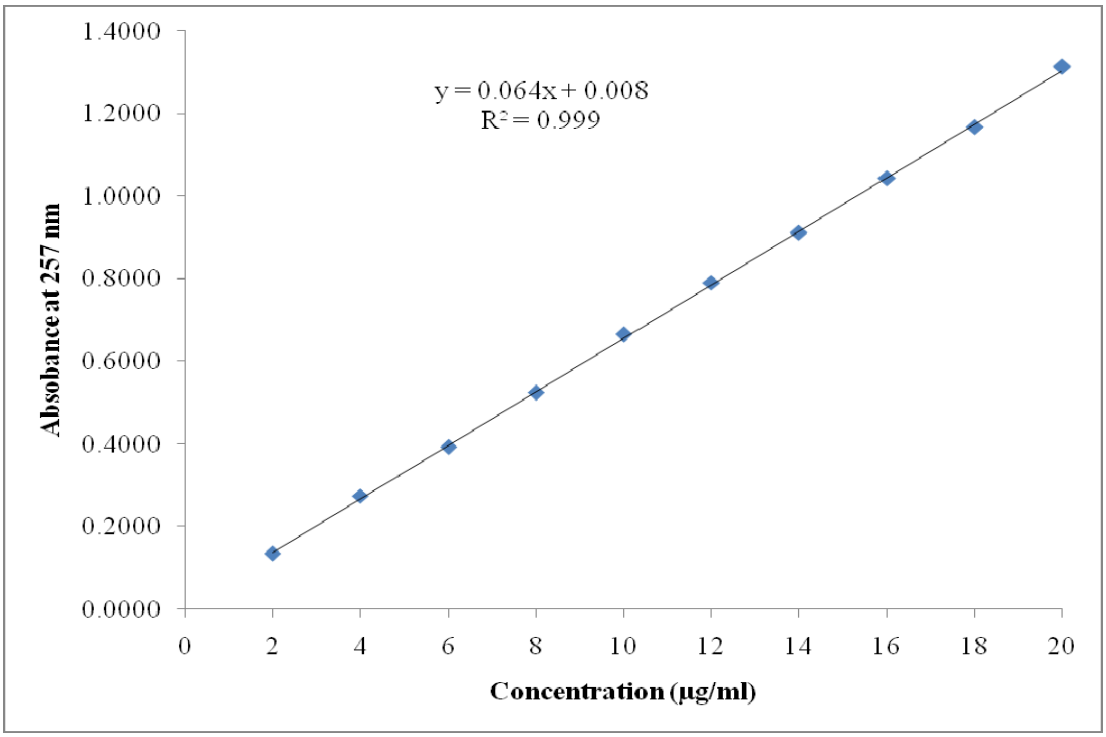



**Figure-2**

**Sulfamethoxazole standard curve using phosphate buffer solution pH 7.4**

**Preparation of combination of Diclofenac sodium and Sulfamethoxazole standard curve using phosphate buffer solution pH 7.4:**

Accurately weighed 50 mg of diclofenac sodium and 100 mg of sulfamethoxazole and transferred to 50 ml SMF, small amount of pH 7.4 was added to dissolve diclofenac sodium, and then the volume was make upto 50 ml with pH 7.4 to get a concentration of 1 mg/ml for diclofenac sodium and 2 mg/ml for sulfamethoxazole. From the above solution 20 ml was pipette out into a 200 ml volumetric flask and made upto the mark using phosphate buffer solution pH 7.4 to get a concentration of 100 µg/ml for diclofenac sodium and 200 µg/ml for. It's a stock solution. From the stock solution, aliquots of 2 ml, 4 ml, 6 ml, 8 ml, 10 ml, 12 ml, 14 ml, 16 ml, 18 ml and 20 ml were pipetted out into a series of 100 ml SMF and made upto the 100 ml mark with phosphate buffer solution pH 7.4 to get drug concentration in the range of 2 to 20 µg/ml for diclofenac sodium. The absorbance of the resulting solution was then measured at 257 nm in a UV double beam spectrophotometer against phosphate buffer solution pH 7.4 as blank. From the stock solution, aliquots of 1 ml, 2 ml, 3 ml, 4 ml, 5 ml, 6 ml, 7 ml, 8 ml, 9 ml, and 10 ml were pipetted out into a series of 100 ml SMF and made upto the 100 ml mark with phosphate buffer solution

pH 7.4 to get drug concentration in the range of 2 to 20 µg/ml for sulfamethoxazole. The absorbance of the resulting solution was then measured at 276 nm in a UV double beam spectrophotometer against phosphate buffer solution pH 7.4 as blank. The standard curve was obtained by plotting concentration (µg/ml) values in X-axis and absorbance values in Y-axis[79].

**Table-4**

**Diclofenac sodium standard curve in combination with Sulfamethoxazole using phosphate buffer solution pH 7.4**

| Concentration (µg/ml) | Diclofenac sodium at 276 nm | | | |
|---|---|---|---|---|
| | 1st time | 2nd time | 3rd time | Average with SD* |
| 2 | 0.134 | 0.122 | 0.114 | 0.1233 ± 0.0101 |
| 4 | 0.277 | 0.249 | 0.25 | 0.2587 ± 0.0159 |
| 6 | 0.411 | 0.368 | 0.374 | 0.3843 ± 0.0233 |
| 8 | 0.526 | 0.511 | 0.496 | 0.5110 ± 0.0150 |
| 10 | 0.654 | 0.618 | 0.625 | 0.6323 ± 0.0191 |
| 12 | 0.762 | 0.734 | 0.754 | 0.7500 ± 0.0144 |
| 14 | 0.867 | 0.865 | 0.877 | 0.8697 ± 0.0064 |
| 16 | 0.998 | 1.02 | 1.016 | 1.0113 ± 0.0117 |
| 18 | 1.136 | 1.135 | 1.136 | 1.1357 ± 0.0006 |
| 20 | 1.271 | 1.235 | 1.283 | 1.2630 ± 0.0250 |

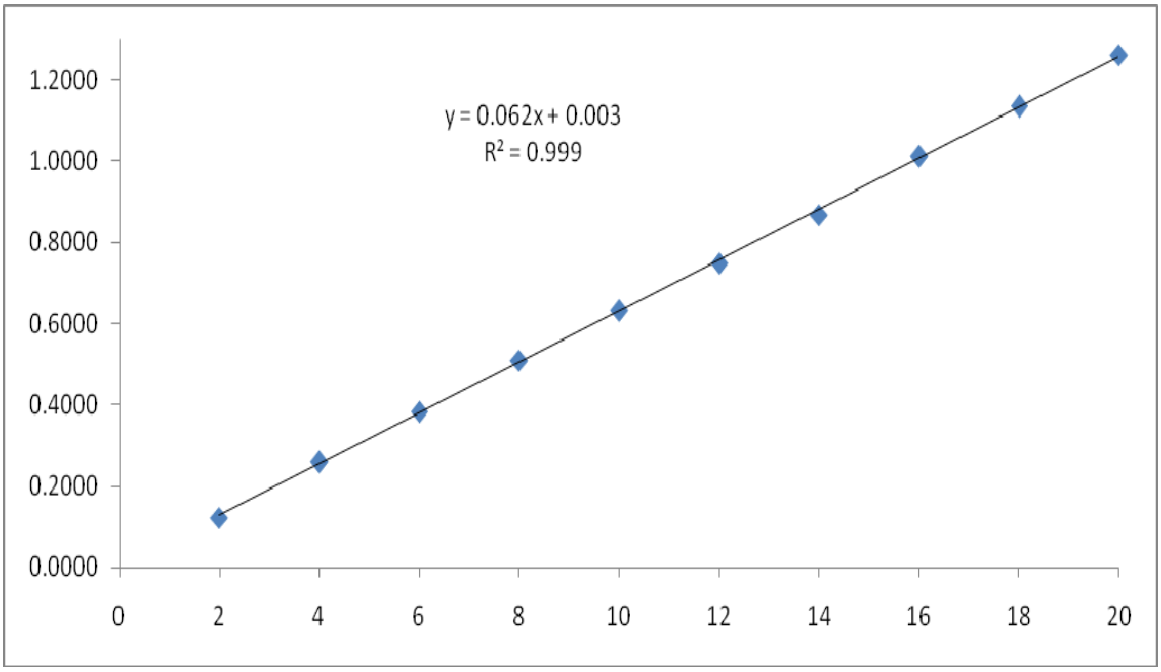



**Figure-3**

**Diclofenac sodium standard curve in combination with Sulfamethoxazole using phosphate buffer solution pH 7.4**

**Table-5**

**Sulfamethoxazole standard curve in combination with Diclofenac sodium using phosphate buffer solution pH 7.4**

| Concentration (µg/ml) | Sulfamethoxazole at 257 nm | | | |
|---|---|---|---|---|
| | 1st time | 2nd time | 3rd time | Average with SD |
| 2 | 0.178 | 0.176 | 0.168 | 0.1740 ± 0.0053 |
| 4 | 0.366 | 0.361 | 0.365 | 0.3640 ± 0.0026 |
| 6 | 0.544 | 0.537 | 0.543 | 0.5413 ± 0.0038 |
| 8 | 0.692 | 0.739 | 0.718 | 0.7163 ± 0.0235 |
| 10 | 0.878 | 0.903 | 0.903 | 0.8947 ± 0.0144 |
| 12 | 1.027 | 1.071 | 1.087 | 1.0617 ± 0.0311 |
| 14 | 1.177 | 1.26 | 1.265 | 1.2340 ± 0.0494 |
| 16 | 1.36 | 1.486 | 1.46 | 1.4353 ± 0.0665 |
| 18 | 1.547 | 1.649 | 1.631 | 1.6090 ± 0.0544 |
| 20 | 1.679 | 1.798 | 1.841 | 1.7727 ± 0.0839 |

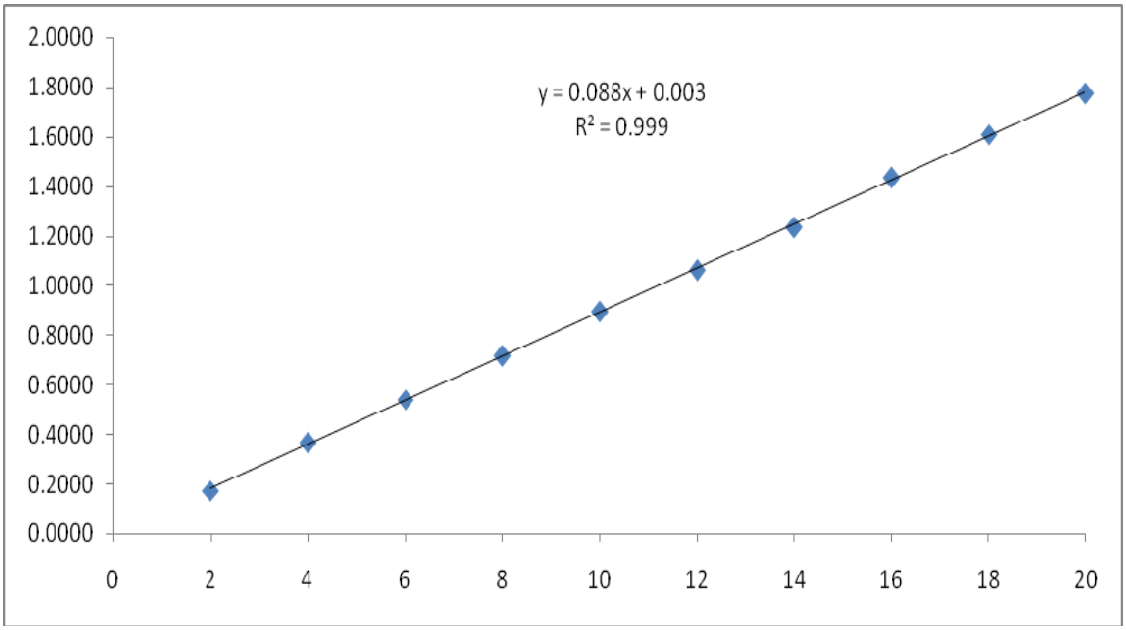



**Figure-4**

**Sulfamethoxazole standard curve in combination with Diclofenac sodium using phosphate buffer solution pH 7.4**

**Preparation of microparticle**

**Preparation of Diclofenac sodium microparticle without chitosan:**

Microparticles containing Diclofenac sodium without chitosan were prepared following cold/hot-dispersion method. Weighed accurately about 6 gm of Poly ethylene glycol-6000 (PEG-6000) in a china dish and allowed to melt in a water bath. When PEG-6000 was completely melted, about 50 mg of diclofenac sodium was added to the molten PEG-6000 and stirred well until diclofenac sodium was dissolved completely.

In 250 ml beaker, 50 ml coconut oil was taken and 100 mg of Span-80 was added and mixed well by using mechanical stirrer for about 5 minutes. Then the melted mixture (PEG-6000+Diclofenac sodium) was added to the coconut oil and span-80 mixture in a thin film. The preparations were allowed to mix in mechanical stirrer for about 1 hour at 800 rpm.

After 1 hr 50 ml of n-hexane was taken in a burette and it was added dropwise to the above preparation for about 45-60 min. with continuous stirring. Then resulting product was filtered, collected and dried at room temperature until it was used further.

**Preparation of Sulfamethoxazole microparticle without chitosan:**

Microparticles containing Sulfamethoxazole without chitosan were prepared following cold/hot-dispersion method. Weighed accurately about 6 gm of PEG-6000 in a china dish and allowed to melt in a water bath. When PEG-6000 was completely melted, about 100 mg of sulfamethoxazole was added to the molten PEG-6000 and stirred well until sulfamethoxazole was dissolved completely.

In 250 ml beaker, 50 ml coconut oil was taken and 100 mg of Span-80 was added and mixed well by using mechanical stirrer for about 5 minutes. Then the melted mixture (PEG-6000+sulfamethoxazole) was added to the coconut oil and span-80 mixture in a thin film. The preparations were allowed to mix in mechanical stirrer for about 1 hour at 800 rpm.

After 1 hr 50 ml of n-hexane was taken in a burette and it was added dropwise to the above preparation for about 45-60 min. with continuous stirring. Then resulting product was filtered, collected and dried at room temperature until it was used further.

**Preparation of Diclofenac sodium and Sulfamethoxazole microparticle without chitosan:**

Microparticles containing Diclofenac sodium and Sulfamethoxazole without chitosan were prepared following cold/hot-dispersion method. Weighed accurately

about 6 gm of PEG-6000 in a china dish and allowed to melt in a water bath. When PEG-6000 was completely melted, about 50 mg of diclofenac sodium and 100 mg of sulfamethoxazole was added to the molten PEG-6000 and stirred well until diclofenac sodium and sulfamethoxazole was dissolved completely.

In 250 ml beaker, 50 ml coconut oil was taken and 100 mg of Span-80 was added and mixed well by using mechanical stirrer for about 5 minutes. Then the melted mixture (PEG-6000+ diclofenac sodium and sulfamethoxazole) was added to the coconut oil and span-80 mixture in a thin film. The preparations were allowed to mix in mechanical stirrer for about 1 hour at 800 rpm.

After 1 hr 50 ml of n-hexane was taken in a burette and it was added dropwise to the above preparation for about 45-60 min. with continuous stirring. Then resulting product was filtered, collected and dried at room temperature until it was used further.

**Preparation of Diclofenac sodium microparticle with chitosan:**

Microparticles containing Diclofenac sodium with chitosan were prepared following cold/hot-dispersion method. Microparticles containing Diclofenac sodium with chitosan were prepared following cold/hot-dispersion method. Weighed accurately about 6 gm of PEG-6000 in a china dish and allowed to melt in a water bath. When PEG-6000 was completely melted, 0.75 ml of propylene glycol was added to the molten PEG-6000 and mixed well. To the above mixture about 75 mg of chitosan was added and allowed to dissolve and then 50 mg of diclofenac sodium was added and stirred well until diclofenac sodium was dissolved completely.

In 250 ml beaker, 50 ml coconut oil was taken and 100 mg of Span-80 was added and mixed well by using mechanical stirrer for about 5 minutes. Then the melted mixture (PEG-6000+ propylene glycol+chitosan+diclofenac sodium) was added to the coconut oil and span-80 mixture in a thin film. The preparations were allowed to mix in mechanical stirrer for about 1 hour at 800 rpm.

After 1 hr 50 ml of n-hexane was taken in a burette and it was added dropwise to the above preparation for about 45-60 min. with continuous stirring. Then resulting product was filtered, collected and dried at room temperature until it was used further.

**Preparation of sulfamethoxazole microparticle with chitosan:**

Microparticles containing Sulfamethoxazole with chitosan were prepared following cold/hot-dispersion method. Weighed accurately about 6 gm of PEG-6000 in a china dish and allowed to melt in a water bath. When PEG-6000 was completely melted, 0.75 ml of propylene glycol was added to the molten PEG-6000 and mixed

well. To the above mixture about 75 mg of chitosan was added and allowed to dissolve and then 50 mg of diclofenac sodium and 100 mg of sulfamethoxazole was added and stirred well until diclofenac sodium and sulfamethoxazole was dissolved completely.

In 250 ml beaker, 50 ml coconut oil was taken and 100 mg of Span-80 was added and mixed well by using mechanical stirrer for about 5 minutes. Then the melted mixture (PEG-6000+ propylene glycol+chitosan+sulfamethoxazole) was added to the coconut oil and span-80 mixture in a thin film. The preparations were allowed to mix in mechanical stirrer for about 1 hour at 800 rpm.

After 1 hr 50 ml of n-hexane was taken in a burette and it was added dropwise to the above preparation for about 45-60 min. with continuous stirring. Then resulting product was filtered, collected and dried at room temperature until it was used further.

**Preparation of diclofenac sodium and sulfamethoxazole microparticle with chitosan:**

Microparticles containing Diclofenac sodium and Sulfamethoxazole with chitosan were prepared following cold/hot-dispersion method. Weighed accurately about 6 gm of PEG-6000 in a china dish and allowed to melt in a water bath. When PEG-6000 was completely melted, 0.75 ml of propylene glycol was added to the molten PEG-6000 and mixed well. To the above mixture about 75 mg of chitosan was added and allowed to dissolve and then 50 mg of diclofenac sodium and 100 mg of sulfamethoxazole was added and stirred well until diclofenac sodium and sulfamethoxazole were dissolved completely.

In 250 ml beaker, 50 ml coconut oil was taken and 100 mg of Span-80 was added and mixed well by using mechanical stirrer for about 5 minutes. Then the melted mixture (PEG-6000+ propylene glycol+chitosan+diclofenac sodium+sulfamethoxazole) was added to the coconut oil and span-80 mixture in a thin film. The preparations were allowed to mix in mechanical stirrer for about 1 hour at 800 rpm.

After 1 hr 50 ml of n-hexane was taken in a burette and it was added dropwise to the above preparation for about 45-60 min. with continuous stirring. Then resulting product was filtered, collected and dried at room temperature until it was used further[80].

**Table-6**

**Microparticle formulations**

| S. No. | Ingredients | F1 | F2 | F3 | F4 | F5 | F6 |
|---|---|---|---|---|---|---|---|
| 1 | PEG-6000 | 6 gm | 6 gm | 6 gm | 6 gm | 6 gm | 6 gm |
| 2 | Diclofenac sodium | 50 mg | - | 50 mg | 50 mg | - | 50 mg |
| 3 | Sulfamethoxazole | - | 100 mg | 100 mg | - | 100 mg | 100 mg |
| 4 | Coconut oil | 50 ml | 50 ml | 50 ml | 50 ml | 50 ml | 50 ml |
| 5 | Span-80 | 100 mg | 100 mg | 100 mg | 100 mg | 100 mg | 100 mg |
| 6 | Chitosan | - | - | - | 75 mg | 75 mg | 75 mg |
| 7 | Propylene glycol | - | - | - | 0.5 ml | 0.5 ml | 0.5 ml |
| 8 | N-hexane | 50 ml | 50 ml | 50 ml | 50 ml | 50 ml | 50 ml |

**Determination of percentage yield:**

The microparticles were dried at room temperature and then it was weighed. The percentage yield for the prepared microparticle was calculated using formula[81]:

$$\text{Percentage Yield} = \frac{\text{Practical yield}}{\text{Theoritical yield}} \times 100$$

**Determination of Drug content:**

Weighed accurately microparticles contain 5 mg of Diclofenac sodium and 10 mg Sulfamethoxazole and combination of 5 mg Diclofenac sodium, 10 mg Sulfamethoxazole was dissolved in buffer pH 7.4. Then the solution was suitably diluted with pH 7.4 and absorbance of the resulting solution was measured at 276 nm for diclofenac sodium and 257 nm for sulfamethoxazole in UV double beam spectrophotometer against phosphate buffer solution pH 7.4 as blank[82]

$$\text{Drug content} = \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}} \times 100$$

**FT-IR Studies**

To determine any interaction between drug and polymer, Fourier Transform Infra Red (FT-IR) study was carried out. The drug and excipients must be compatible with one another to produce a stable, efficacious, easy to administer and safe product. FT-IR analysis of pure drug and drug +excipients was studied[83].

**Sieving method procedure:**

Standard sieve sets of various sizes was selected (sieve no. 10; 22; 36; 44; 65; 80; 100 & 120). The coarsest sieve (sieve no. 10) is arranged at the top and the finest sieve (sieve no.120) was arranged at the bottom. The pan was kept below the sieve no.120. A known weight of 10 gm microparticles was weighed and placed on the coarsest sieve (sieve no.10). The sieve set was shaken for 15 min. The microparticles retained on each sieves was collected separately. Weighed the microparticles retained on each sieves[84].

**In-Vitro drug release studies:**

In-vitro drug release was studied using USP II apparatus type 1 (basket), with 900ml of dissolution medium maintained at 37±2°C at 50 rpm by using phosphate buffer of pH 7.4 as a dissolution medium. 1ml of sample was withdrawn in different time intervals, and the sample was replaced by an equal volume of fresh dissolution medium of same pH to maintain the sink condition. The samples were analyzed spectrophotometrically at 276 nm Diclofenac sodium and 257 nm for Sulfamethoxazole, and the percent drug released was calculated[85].

**Gel preparation procedure:**

Weighed accurately (0.5% w/v, 0.75% w/v, 1.00% w/v, 1.25% w/v and 1.5% w/v) specific quantity of carbomer. Some amount of distilled water was taken in a beaker and to that the weighed amount of carbomer was slowly added (in order to prevent the agglomeration of particle) to the beaker containing water. It was mixed well with the help of magnetic stirrer at 250 rpm. Care must be taken while mixing to prevent the formation of air bubbles in the gel. Mixing was continued until a homogeneous mixture was obtained. Then the volume was make upto 100 ml with distilled water and mixed well.

**Gel evaluation procedure:**

**Spreadability test:**

1 gm of gel was weighed and taken in one pre-weighed glass slide to that another glass slide was placed over it. The two glass slides were compressed by placing a 100 gm weight over it and leave it for 60 sec. for uniform spreading of the gel. After 60 sec. the excess gel on the side of the slide was wiped. One glass slide was fixed and another one was movable. The movable glass slide was allowed to move for a distance of 7.5 cm and the time was noted. Lesser the time taken for the

slide to cross the 7.5 cm distance shows better spreadability. The study was performed in triplicate[86].

**Consistency test:**

Plastic collapsible tubes filled with (0.5% w/v, 0.75% w/v, 1.00% w/v, 1.25% w/v and 1.5% w/v) 10 gm of gel. A tube was compressed and extrudibility of the formulation was determined in terms of weight in grams applied over the tube. Weight required to extrude a 0.5 cm. ribbon of gel in 10 seconds[87].

**Microparticles mixed with gel procedure:**

Accurately weighed microparticle (F1, F2, F3, F4, F5 and F6) and 500 mg of gel (0.75% w/v and 1.00%w/v) was added slowly in beaker containing magnetic stirrer and mixed in magnetic stirrer for 5 min. at 210 rpm.

**Permeation studies procedure:**

A cellophane membrane, (soaked in glycerin 12 hours before use) was fixed to one end of the two side open ended cylinder (donor compartment). The sample was taken in a cellophane membrane and it was immersed in a beaker containing 100 ml of phosphate buffer pH 7.4 (receptor compartment). The entire surface of the cellophane membrane was in contact with the receptor compartment which was agitated using magnetic stirrer rotated at 200 rpm and a temperature of 37°C±2°C was maintained. 1 ml of samples from the receptor compartment was taken at every 5 min. interval for 30 min. then 45 and 60 min. and then every 30 min. interval for upto 6 hrs., same amount of pH 7.4 was replaced. The samples were suitably diluted with pH 7.4. The absorbance of the resulting solution was then measured at 276 nm for Diclofenac sodium and 257 nm for Sulfamethoxazole using UV double beam spectrophotometer against phosphate buffer solution pH 7.4 as blank[88].

**Release Kinetics**

To know the release kinetics, the value obtained from *in vitro* drug release studies were plotted in various kinetic models like zero order, first order, higuchi model hixson-crowell and korsmeyer–peppas.

**Zero order**

Zero order is Cumulative amount of drug release vs time.

Equation is $C=K_0t$,

where

$K_0$ is the zero order rate constant expressed in units of concentration/time and t is the time in hours.

**First order**

First order is Log cumulative percentage drug remaining vs time.

Equation is $\text{Log } C = \text{Log } C_0 - K_t/2.303$,

where

$C_0$ is the initial concentration of drug,

K is the first order constant and t is the time.

**Higuchi model**

Higuchi model is the Cumulative percent drug release vs square root of time.

Equation is $Q = K_t 1/2$,

where

K is the constant reflecting the design variables of the system and

t is the time in hours.

**Hixson-Crowell model**

Hixson-Crowell model is the cube root percent drug remaining vs time.

Equation is $\sqrt[3]{Q_0} - \sqrt[3]{Q_t} = KHCXt$,

where,

$Q_0$ is the initial amount of drug in the tablet,

$Q_t$ is the amount of drug release in time t,

KHC is the ration constant for the Hixson-Crowell rate equation and

t is the time.

**Korsmeyer–Peppas model**

Korsmeyer peppas is the log cumulative percentage of drug release vs log time.

The exponent n was calculated through the slope of straight line.

$$M_t/M_\infty = ktn$$

Where,

$M_t/M_\infty$ is the fractional solute release,

K is the kinetic constant characteristic of the drug polymer system,

t is the release time and

n is the exponent.

# *Results and Discussion*

## RESULTS AND DISCUSSION

**Preformulation studies for pure drug**

**Description**

**Table-7**

**Description of pure Diclofenac sodium**

| Properties | Specification | Observation |
|---|---|---|
| Colour | White or slightly yellowish | White |
| Physical nature | Crystalline powder | Crystalline powder |
| Taste | Bitter | Bitter |
| Odour | Odourless | Odourless |

**Corollary:**

Colour, physical nature, taste and odour of Diclofenac sodium were observed and their result in table-7 shows same as the specification.

**Table-8**

**Description of pure Sulfamethoxazole**

| Properties | Specification | Observation |
|---|---|---|
| Colour | White or almost white | White |
| Physical Nature | Crystalline powder | Crystalline powder |
| Taste | Metallic | Metallic |
| Odour | Unpleasant | Unpleasant |

**Corollary:**

Colour, physical nature, taste and odour of Sulfamethoxazole were observed and their result shown in table-8 shows same as the specification.

**Solubility studies**

**Table-9**

**Solubility analysis of Diclofenac sodium and Sulfamethoxazole**

| Solubility | Specification/ Observation | Diclofenac sodium | Sulfamethoxazole |
|---|---|---|---|
| **Water** | **Specification** | Sparingly soluble | Practically insoluble |
| | **Observation** | Sparingly soluble | Insoluble |
| **Methanol** | **Specification** | Freely soluble | Freely soluble |
| | **Observation** | Freely soluble | Freely soluble |
| **Ethanol (96%)** | **Specification** | Freely soluble | Sparingly soluble |
| | **Observation** | Freely soluble | Sparingly soluble |
| **Acetone** | **Specification** | Slightly soluble | Freely soluble |
| | **Observation** | Slightly soluble | Freely soluble |

**Corollary:**

Solubility studies of pure Diclofenac sodium and Sulfamethoxazole were observed based on the procedure and the reports were shown in table-9. The solubility of Diclofenac sodium and Sulfamethoxazole was found to be same as the specification.

**Physical properties**

**Table-10**

**Melting point analysis of Diclofenac sodium and Sulfamethoxazole**

| Properties | Melting point ranges | Observed value |
|---|---|---|
| **Diclofenac sodium** | About 280 °C, with decomposition | Degradation started at 280°C |
| **Sulfamethoxazole** | 169°C to 172°C | 169°C |

**Corollary:**

The observed value for melting point of the both drugs was found to be within the range of the monograph and the values are shown in table-10.

**Table-11**

**Physical observation of drug:excipients compatibility studies**

| S. No. | Drug and excipients | Parameter |
|---|---|---|
| 1. | Diclofenac sodium | No Characteristic change |
| 2. | Sulfamethoxazole | No Characteristic change |
| 3. | Diclofenac sodium + Sulfamethoxazole | No Characteristic change |
| 4. | Diclofenac sodium + Excipients | No Characteristic change |
| 5. | Sulfamethoxazole + Excipients | No Characteristic change |
| 6. | Diclofenac sodium + Sulfamethoxazole + Excipients | No Characteristic change |

**Corollary:**

The drug excipients compatibility from the table-11 showed that there was no change or interaction between drug and excipients. Thus it was concluded that the excipients selected for the formulation were compatible with pure drug.

**Determination of Percentage Yield:**

The microparticles were dried at room temperature and then it was weighed.

**Table-12**

**Percentage yield for the prepared microparticle formulations**

| Formulations | Percentage yield |
|---|---|
| F1 | 95.76 |
| F2 | 94.13 |
| F3 | 95.12 |
| F4 | 96.87 |
| F5 | 96.20 |
| F6 | 97.18 |

**Corollary:**

The percentage yield for the prepared microparticle was found to be in the range 94.13 to 97.18. The results were tabulated in table-12.

**Determination of Drug content**

The drug content for all the prepared microparticle formulations (F1 to F6) were determined by dissolving the microparticle in pH 7.4 and diluted suitably with the same solvents. The resulting solution was measured at 276 nm for diclofenac

sodium and 257 nm for sulfamethoxazole in UV double beam spectrophotometer against phosphate buffer solution pH 7.4 as blank.

**Table-13**

**Determination of drug content**

| Formulations | Drug content |
|---|---|
| F1 | 95.04 |
| F2 | 95.52 |
| F3 | Diclo-96.76 |
| | Sulfa-96.95 |
| F4 | 95.23 |
| F5 | 95.55 |
| F6 | 96.61 |
| | 96.88 |

**Corollary:**

The drug content of diclofenac sodium was found to be in the range of 95.04% to 96.76 % and for sulfamethoxazole it was 95.52% to 96.95%. The results were shown in table

**FT-IR Studies**

The FT-IR studies were performed to determine the interaction between drug and drug, drug and polymer. The results were shown in table-14 to and figure-5 to 13.

**Figure-5**

**FT-IR for pure Diclofenac sodium**

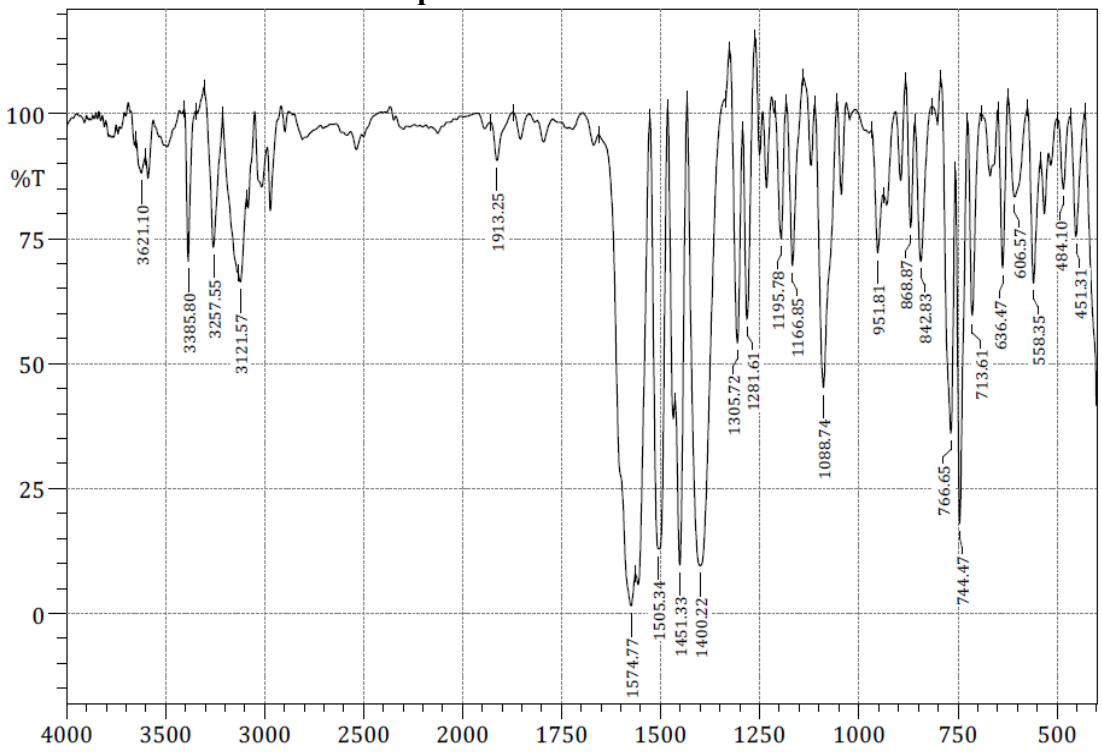



**Table-14**

**FT-IR for pure Diclofenac sodium**

| S. No. | Wave number ($Cm^{-1}$) | Functional group |
|---|---|---|
| 1. | 3163.04 | NH stretching of secondary amine |
| 2. | 3121.57 | C-H stretching aromatic compound |
| 3. | 1913.25 | C=O stretching in ester |
| 4. | 1574.77 | C=O stretching carboxyl ion |
| 5. | 1505.34 | C=C stretching aromatic compound |
| 6. | 1305.72 | C-N stretching group |
| 7. | 1281.61 | C-N stretching group |
| 8. | 744.47 | C-Cl stretching group |

**Figure-6**

**FT-IR for pure Sulfamethoxazole**

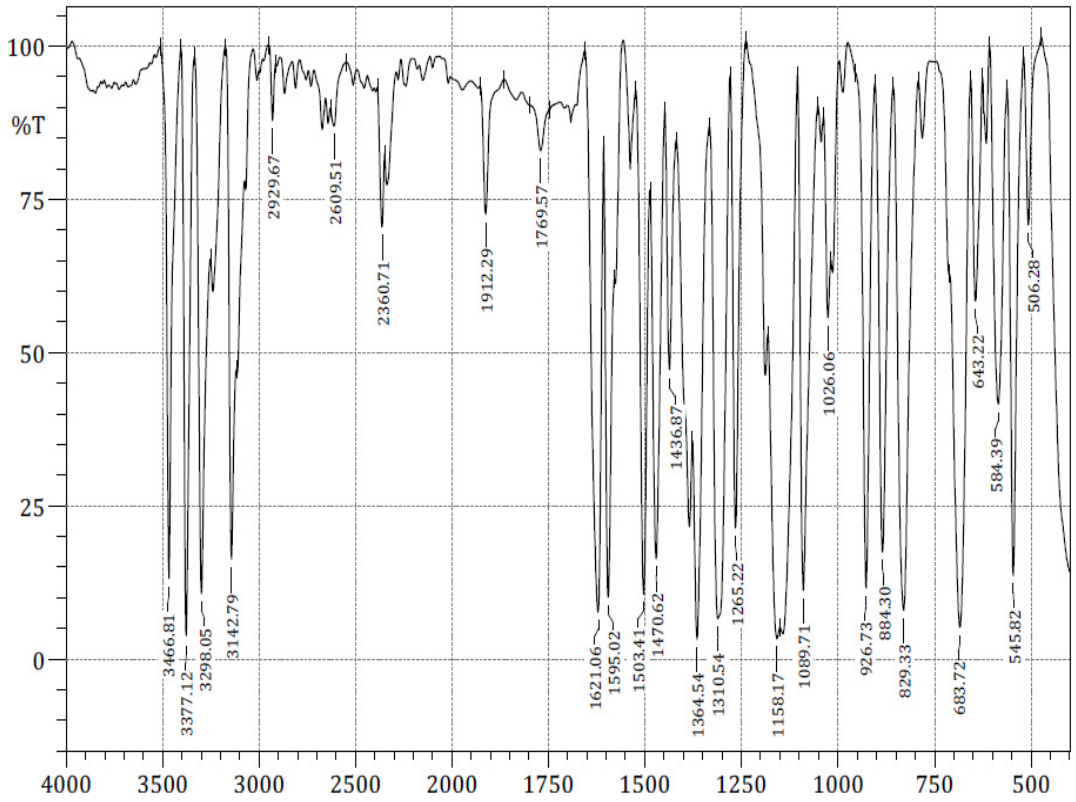



**Table-15**

**FT-IR for pure Sulfamethoxazole**

| S. No. | Wave number ($Cm^{-1}$) | Functional group |
|---|---|---|
| 1. | 3298.05 | NH stretching of group |
| 2. | 3142.79 | C-H stretching aromatic group |
| 3. | 2929.67 | C-H stretching aliphatic group |
| 4. | 2609.51 | C=O stretching carboxyl ion |
| 5. | 1769.57 | C-N stretching in oxazole group |
| 6. | 1621.06 | C-C stretching group |
| 7. | 1503.41 | C=N stretching in oxazole group |
| 8. | 1470.62 | C=C stretching aromatic group |
| 9. | 1364.54 | S=O stretching group |
| 10. | 1158.17 | C-N symmetric SO2 stretching group |

**Figure-7**

**FT-IR Combination for Diclofenac sodium and Sulfamethoxazole**

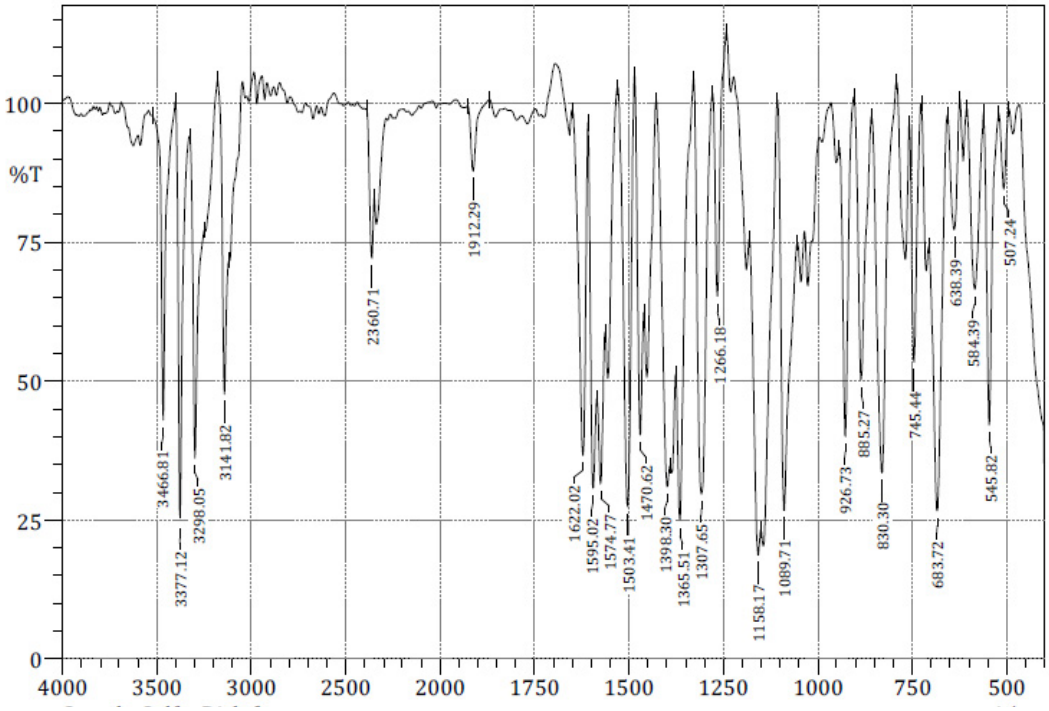



**Table-16**

**FT-IR Combination drug for Diclofenac sodium and Sulfamethoxazole**

| S. No. | Wave number ($Cm^{-1}$) | Functional group |
|---|---|---|
| 1. | 3141.82 | NH stretching aromatic of group |
| 2. | 1913.25 | C=O stretching in ester group |
| 3. | 1574.77 | C=O stretching of carboxylic ion group |
| 4. | 1503.41 | C=C stretching aromatic group |
| 5. | 1470.62 | C=N stretching in oxazole group |
| 6. | 1365.51 | S=O stretching assymetric group |
| 7. | 1307.64 | C-N stretching group |
| 8. | 1158.17 | Symmetric SO2 stretching group |
| 9. | 754.44 | C-Cl stretching Group |

**Figure-8**

**FT-IR for F1 formulation**

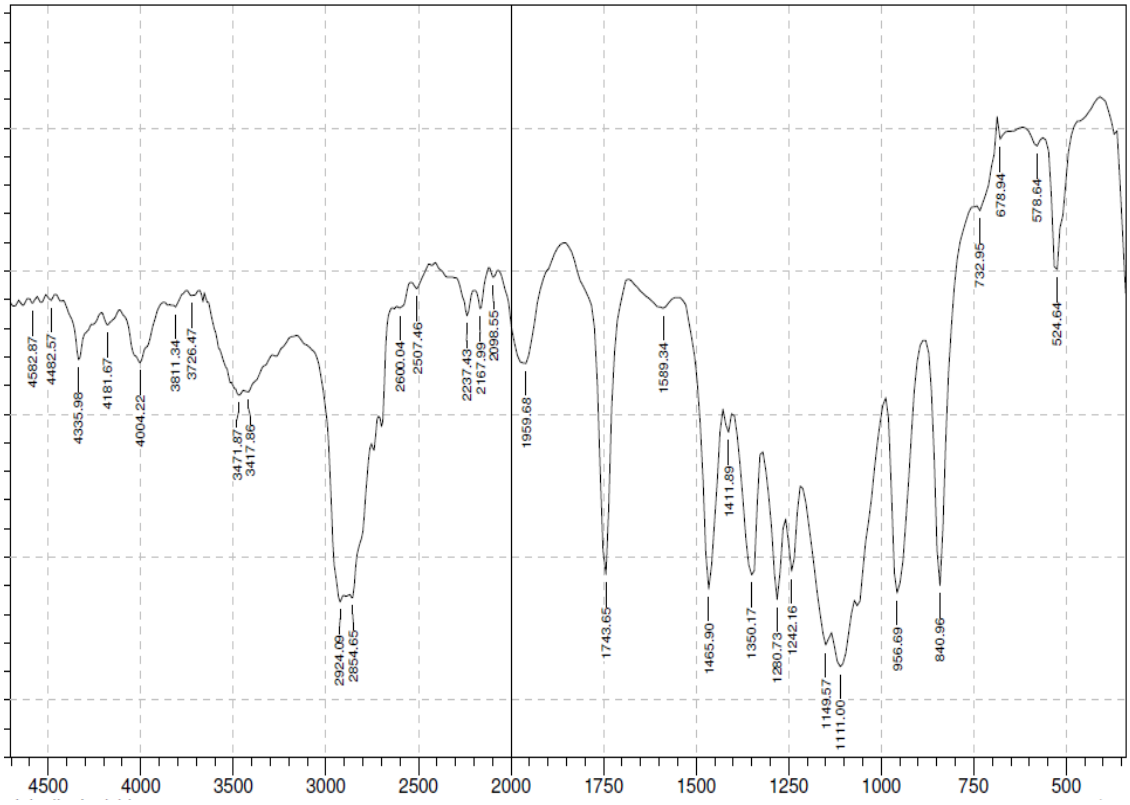



**Table-17**

**FT-IR for F1 formulation**

| S. No. | Wave number ($Cm^{-1}$) | Functional Group |
|---|---|---|
| 1. | 3471.87 | Amine N-H stretching group. |
| 2. | 2854.65 | Alkane C-H stretching group |
| 3. | 1959.68 | Aromatic C-H Group |
| 4. | 1743.65 | Carboxylic C=O stretching group |
| 5. | 1465.90 | Aromatic C-H stretching group |
| 6. | 1111.00 | C-O stretching group |
| 7. | 840.96 | C-Cl stretching group |

**Figure-9**

**FT-IR for F2 formulation**

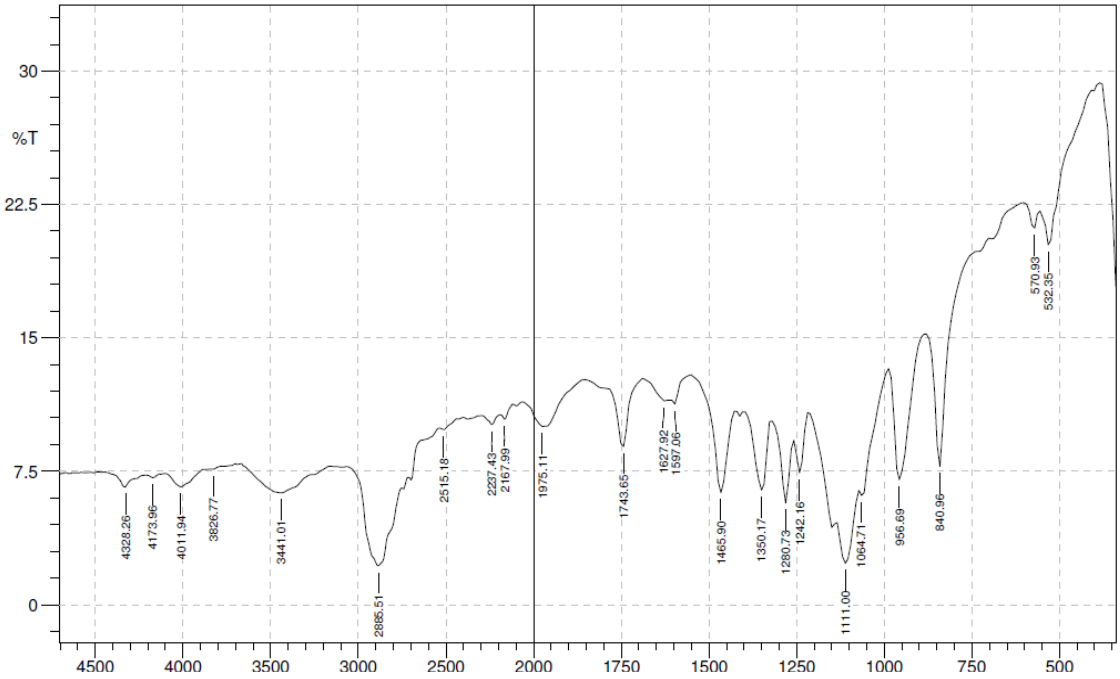



**Table-18**

**FT-IR for F2 formulation**

| S. No. | Wave number ($Cm^{-1}$) | Functional Group |
|---|---|---|
| 1. | 2885.51 | Alkane C-H stretching group |
| 2. | 19759.11 | Aromatic C-H Group |
| 3. | 1743.65 | Carboxylic C=O stretching group |
| 4. | 1465.90 | Aromatic C-H stretching group |
| 5. | 1111.00 | C-O stretching group |
| 6. | 840.96 | C-Cl stretching group |

**Figure-10**

**FT-IR for F3 formulation**

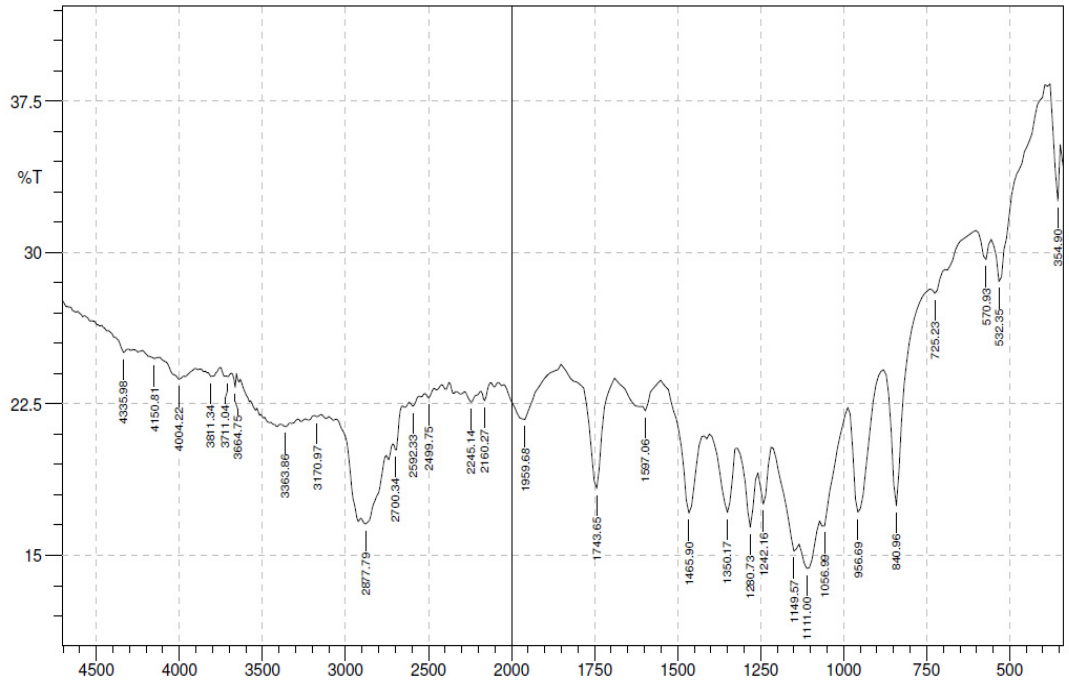



**Table-19**

**FT-IR for F3 formulation**

| S. No. | Wave number ($Cm^{-1}$) | Functional Group |
|---|---|---|
| 1. | 2877.79 | Alkane C-H stretchin group |
| 2. | 1959.68 | Aromatic C-H Group |
| 3. | 1743.65 | CarboxylicC=O stretchin group |
| 4. | 1465.90 | Aromatic C-H stretching group |
| 5. | 1111.00 | C-O stretching group |
| 6. | 840.96 | C-Cl stretching group |

**Figure-11**

**FT-IR for F4 formulation**

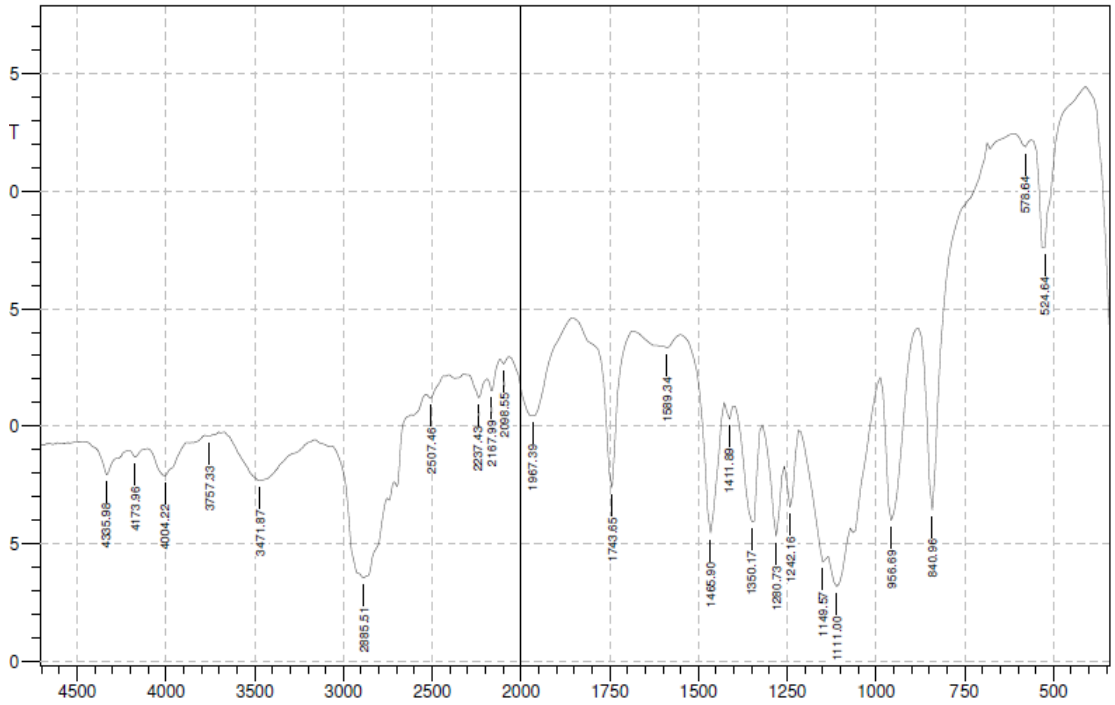



**Table-20**

**FT-IR for F4 formulation**

| S. No. | Wave number ($Cm^{-1}$) | Functional Group |
|---|---|---|
| 1. | 3471.87 | Amine N-H stretching group. |
| 2. | 2885.51 | Alkane C-H stretching group |
| 3. | 1967.39 | Aromatic C-H Group |
| 4. | 1743.65 | Carboxylic C=O stretching group. |
| 5. | 1465.90 | Aromatic C-H stretching group. |
| 6. | 1111.00 | C-O stretching group |
| 7. | 840.96 | C-Cl stretching group |

**Figure-12**

**FT-IR for F5 formulation**

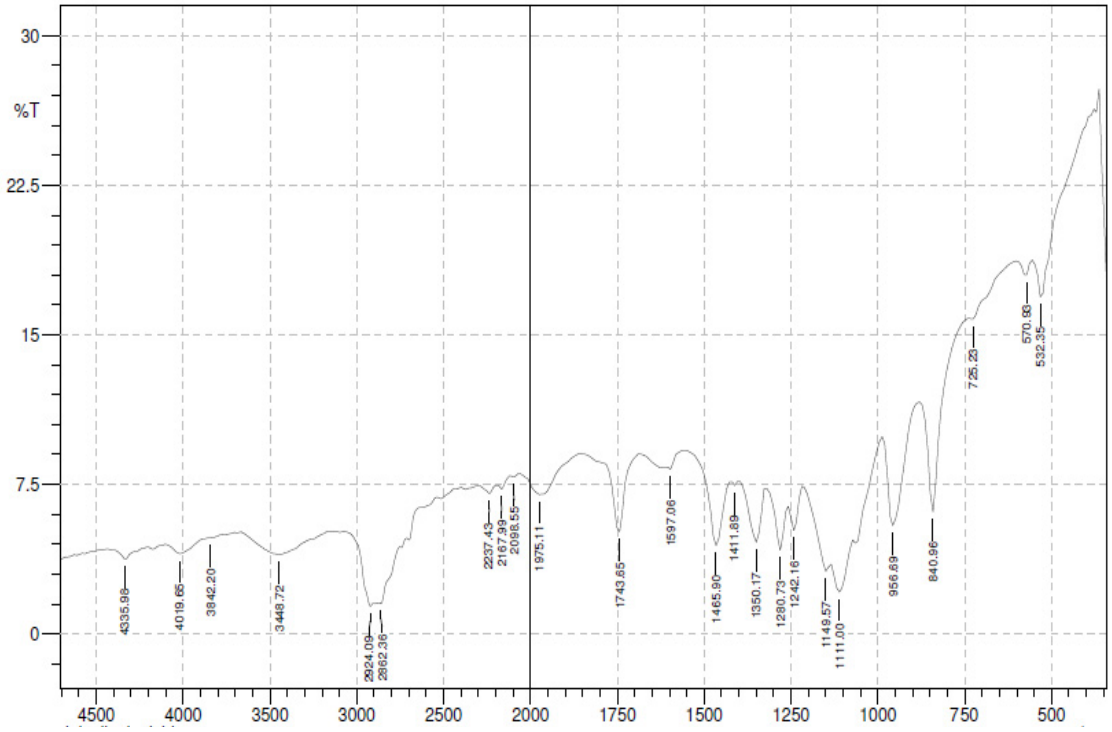



**Table-21**

**FT-IR for F5 formulation**

| S. No. | Wave number ($Cm^{-1}$) | Functional Group |
|---|---|---|
| 1. | 2862.36 | Alkane C-H stretching group |
| 2. | 1975.11 | Aromatic C-H Group |
| 3. | 1743.65 | Carboxyli C=O stretching group. |
| 4. | 1465.90 | Aromatic C-H stretching group |
| 5. | 1111.00 | C-O stretching group |
| 6. | 840.96 | C-Cl stretching group |

**Figure-13**

**FT-IR for F6 formulation**

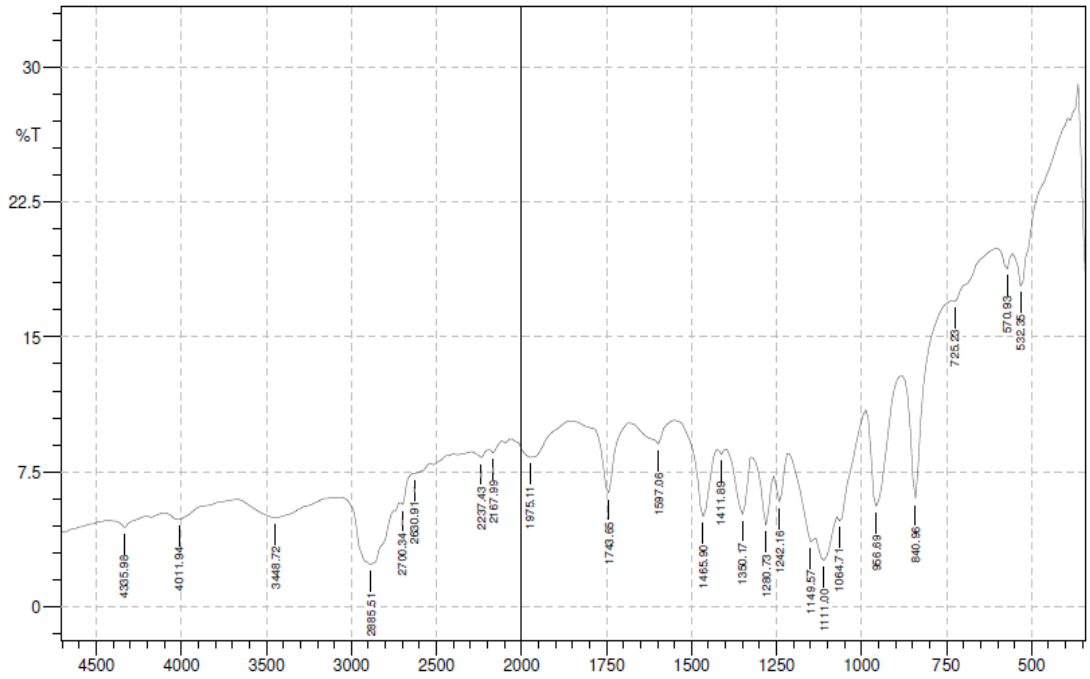



**Table-22**

**FT-IR for F6 formulation**

| S.No. | Wave number ($Cm^{-1}$) | Functional Group |
|---|---|---|
| 1. | 2885.51 | Alkane C-H stretching group |
| 2. | 1975.11 | Aromatic C-H Group |
| 3. | 1743.65 | CarboxylicC=O stretching group |
| 4. | 1465.90 | Aromatic C-H stretching group |
| 5. | 1111.00 | C-O stretching group |
| 6. | 840.96 | C-Cl stretching group |

**Table-23**

**FT – IR Spectrum of pure Diclofenac sodium, Sulfamethoxazole, Combination drug Diclofenac sodium + Sulfamethoxazole, F1 formulation, F2 formulation, F3 formulation, F4 formulation, F5 formulation and F6 formulation**

| Functional Group | Amine N-H stretching | Alkane C-H stretching | Carboxylic C=O stretching | Aromatic C-H stretching | C-O stretching | C-Cl stretching |
|---|---|---|---|---|---|---|
| Diclofenac Sodium | 3385.8 | - | 1574.77 | 3121.57 | - | 744.47 |
| Sulfamethoxazole | 3298.05 | - | 2609.51 | 3142.79 | 1436.87 | - |
| Diclofenac Sodium + Sulfamethoxazole | - | - | 1574.77 | 3141.82 | 1913.25 | 754.44 |
| F1 formulation | 3471.87 | 2854.65 | 1743.65 | 1465.90 | 1111.00 | 840.96 |
| F2 formulation | - | 2885.51 | 1743.65 | 1465.90 | 1111.00 | 840.96 |
| F3 formulation | - | 2885.51 | 1743.65 | 1465.90 | 1111.00 | 840.96 |
| F4 formulation | 3471.87 | 2885.51 | 1743.65 | 1465.90 | 1111.00 | 840.96 |
| F5 formulation | 3448.72 | 2862.36 | 1743.65 | 1465.90 | 1111.00 | 840.96 |
| F6 formulation | 3448.72 | 2862.36 | 1743.65 | 1465.90 | 1111.00 | 840.96 |

**Particle size determination by sieving method**

The size of prepared microparticle formulations (F1 to F6) the size of the microparticles were determined by sievieng method. The results were shown in table-24 to 30 and figure-14 to 19.

**Table-24**

**Particle size of F1 formulations by sieving method**

| Sieve number | Aperture sieve size as per IP specification (µm) | Arithmetic mean size of opening (d) µm | Weight of granules retained on a sieve (over size) gm | % weight of granules retained (under size) (n) | Cumulative % of granules retained (under size) | Weight size (n x d) | % under size | % under size | % over size (100 - % under size) |
|---|---|---|---|---|---|---|---|---|---|
| 10 | 1700 | 1205 | 0 | 0 | 0 | 0 | 0 | 0 | 100 |
| 22 | 710 | 922.5 | 3.51 | 35.1 | 35.1 | 32379.75 | 32379.8 | 32.3798 | 67.6203 |
| 36 | 425 | 602.5 | 2.7 | 27 | 62.1 | 16267.5 | 48647.3 | 48.6473 | 51.3528 |
| 44 | 355 | 302.5 | 2.2 | 22 | 84.1 | 6655 | 55302.3 | 55.3023 | 44.6978 |
| 60 | 250 | 215 | 1.05 | 10.5 | 94.6 | 2257.5 | 57559.8 | 57.5598 | 42.4403 |
| 85 | 180 | 165 | 0.48 | 4.8 | 99.4 | 792 | 58351.8 | 58.3518 | 41.6483 |
| 100 | 150 | 137.5 | 0.06 | 0.6 | 100 | 82.5 | 58434.3 | 58.4343 | 41.5658 |
| 120 | 125 | 62.5 | 0 | 0 | 100 | 0 | 58434.3 | 58.4343 | 41.5658 |
| Pan | 0 | 0 | | 0 | 100 | 0 | 58434.3 | 58.4343 | 41.5658 |
| | | | 10 | ∑n = 100 | | ∑(nxd) = 58434.25 | | | |

**Figure-14**

**Particle size of F1 formulations by sieving method**

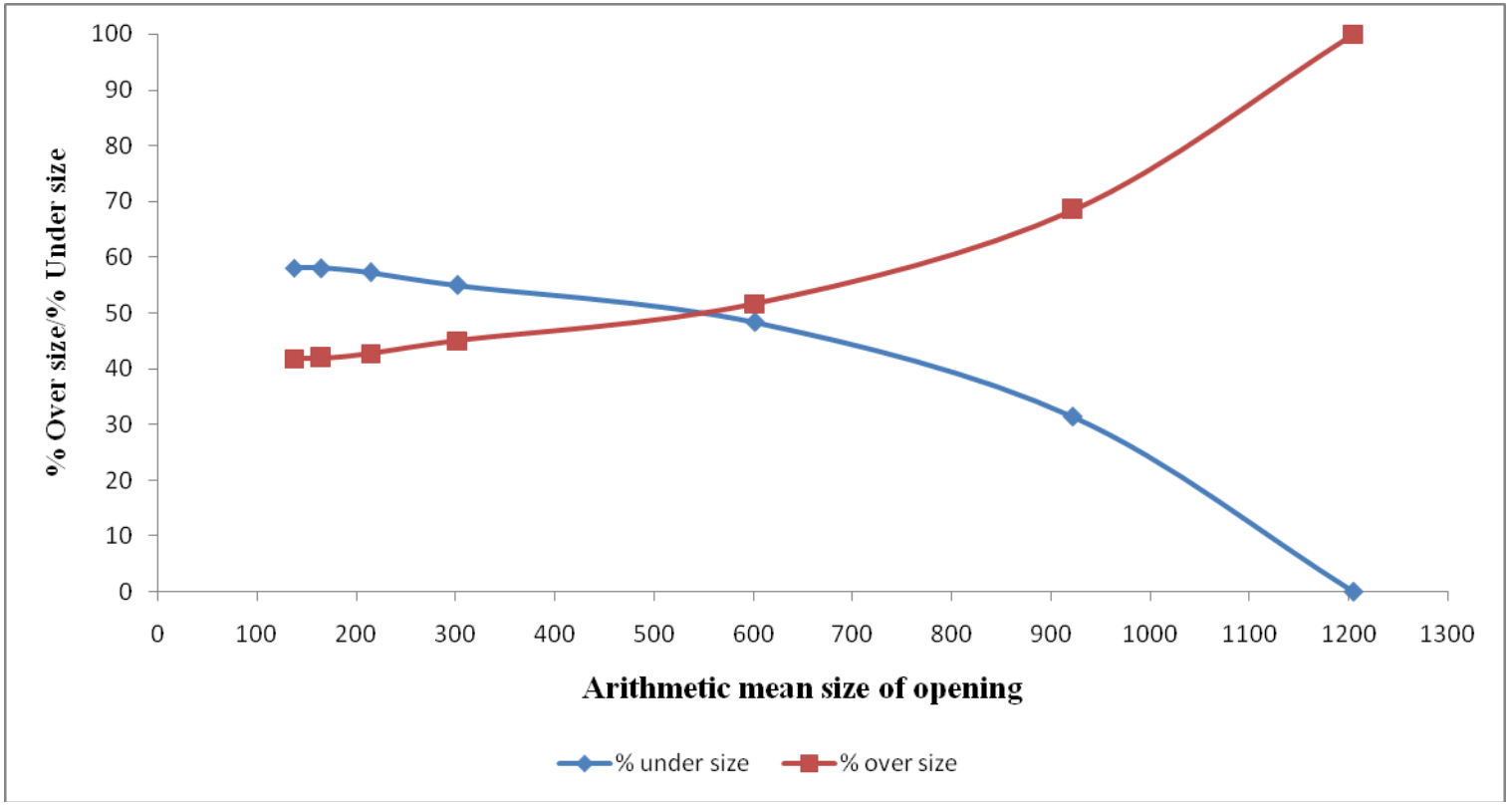


**Table-25**

**Particle size of F2 formulations by sieving method**

| Sieve number | Aperture sieve size as per IP specification (µm) | Arithmetic mean size of opening (d) µm | Weight of granules retained on a sieve (over size) gm | % weight of granules retained (under size) (n) | Cumulative % of granules retained (under size) | Weight size (n x d) | % under size | % under size | % over size (100 - % under size) |
|---|---|---|---|---|---|---|---|---|---|
| 10 | 1700 | 1205 | 0 | 0 | 0 | 0 | 0 | 0 | 100 |
| 22 | 710 | 922.5 | 3.425 | 34.25 | 34.25 | 31595.625 | 31595.625 | 31.595625 | 68.404375 |
| 36 | 425 | 602.5 | 2.875 | 28.75 | 63 | 17321.875 | 48917.5 | 48.9175 | 51.0825 |
| 44 | 355 | 302.5 | 2.1 | 21 | 84 | 6352.5 | 55270 | 55.27 | 44.73 |
| 60 | 250 | 215 | 1.025 | 10.25 | 94.25 | 2203.75 | 57473.75 | 57.47375 | 42.52625 |
| 85 | 180 | 165 | 0.475 | 4.75 | 99 | 783.75 | 58257.5 | 58.2575 | 41.7425 |
| 100 | 150 | 137.5 | 0.1 | 1 | 100 | 137.5 | 58395 | 58.395 | 41.605 |
| 120 | 125 | 62.5 | 0 | 0 | 100 | 0 | 58395 | 58.395 | 41.605 |
| Pan | 0 | 0 | | 0 | 100 | 0 | 58395 | 58.395 | 41.605 |
| | | | 10 | ∑n=100 | | ∑(nxd) = 58395 | | | |

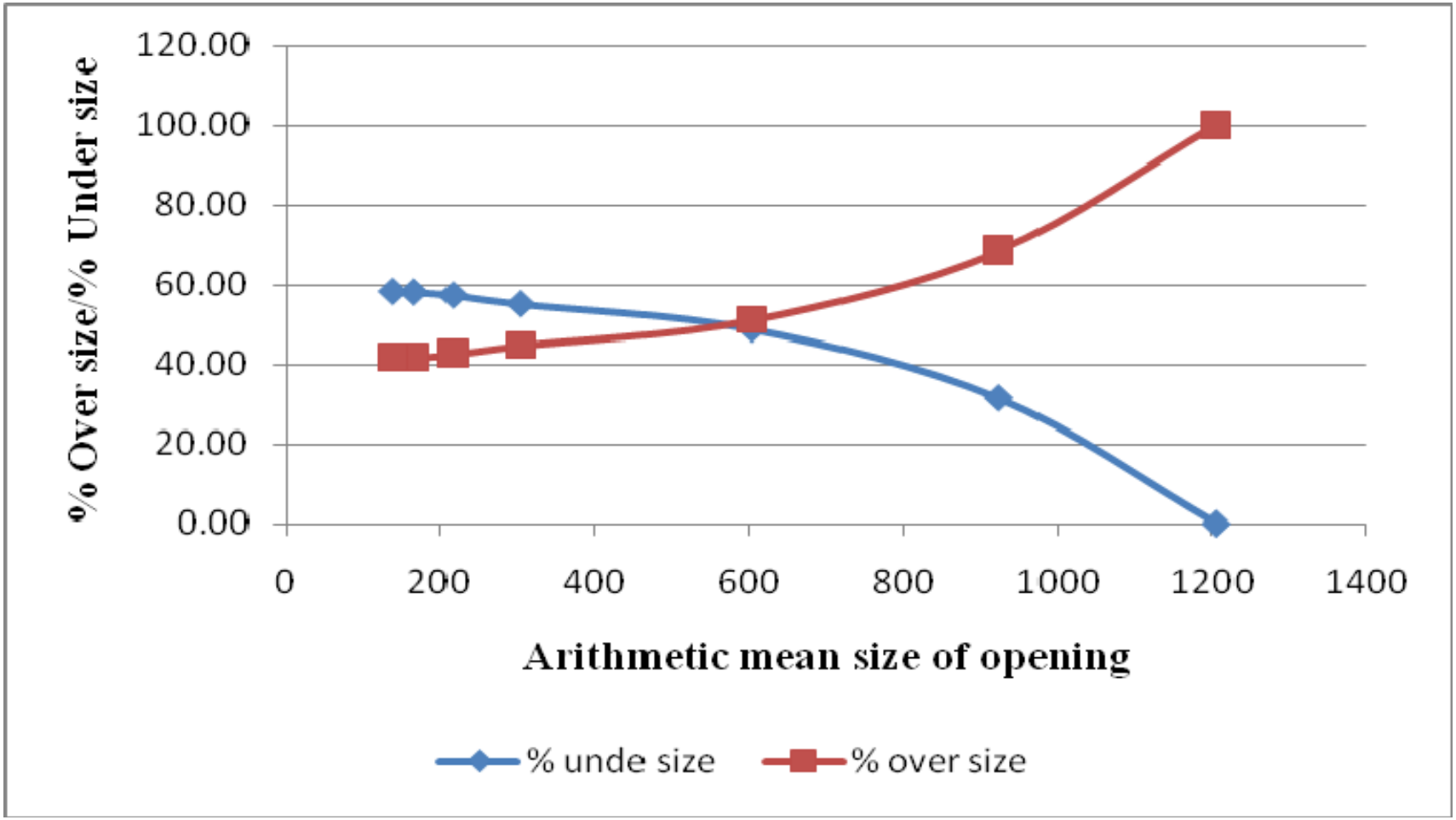



**Figure-15**

**Particle size of F2 formulations by sieving method**

**Table-26**

**Particle size of F3 formulations by sieving method**

| Sieve number | Aperture sieve size as per IP specification (µm) | Arithmetic mean size of opening | Weight of granules retained on a sieve (over size) gm | % weight of granules retained (under size) | Cumulative % of granules retained (under size) | Weight size | % under size | % under size | % over size |
|---|---|---|---|---|---|---|---|---|---|
| | | (d) µm | | (n) | | (n x d) | | | (100 - % under size) |
| 10 | 1700 | 1205 | 0 | 0 | 0 | 0 | 0 | 0 | 100 |
| 22 | 710 | 922.5 | 3.63 | 36.3 | 36.3 | 33486.75 | 33486.8 | 33.4868 | 66.5133 |
| 36 | 425 | 602.5 | 2.75 | 27.5 | 63.8 | 16568.75 | 50055.5 | 50.0555 | 49.9445 |
| 44 | 355 | 302.5 | 1.85 | 18.5 | 82.3 | 5596.25 | 55651.8 | 55.6518 | 44.3483 |
| 60 | 250 | 215 | 1.12 | 11.2 | 93.5 | 2408 | 58059.8 | 58.0598 | 41.9403 |
| 85 | 180 | 165 | 0.6 | 6 | 99.5 | 990 | 59049.8 | 59.0498 | 40.9503 |
| 100 | 150 | 137.5 | 0.05 | 0.5 | 100 | 68.75 | 59118.5 | 59.1185 | 40.8815 |
| 120 | 125 | 62.5 | 0 | 0 | 100 | 0 | | | |
| Pan | 0 | 0 | | 0 | 100 | 0 | | | |
| | | | 10 | ∑n=100 | | ∑(nxd) = 59118.5 | | | |

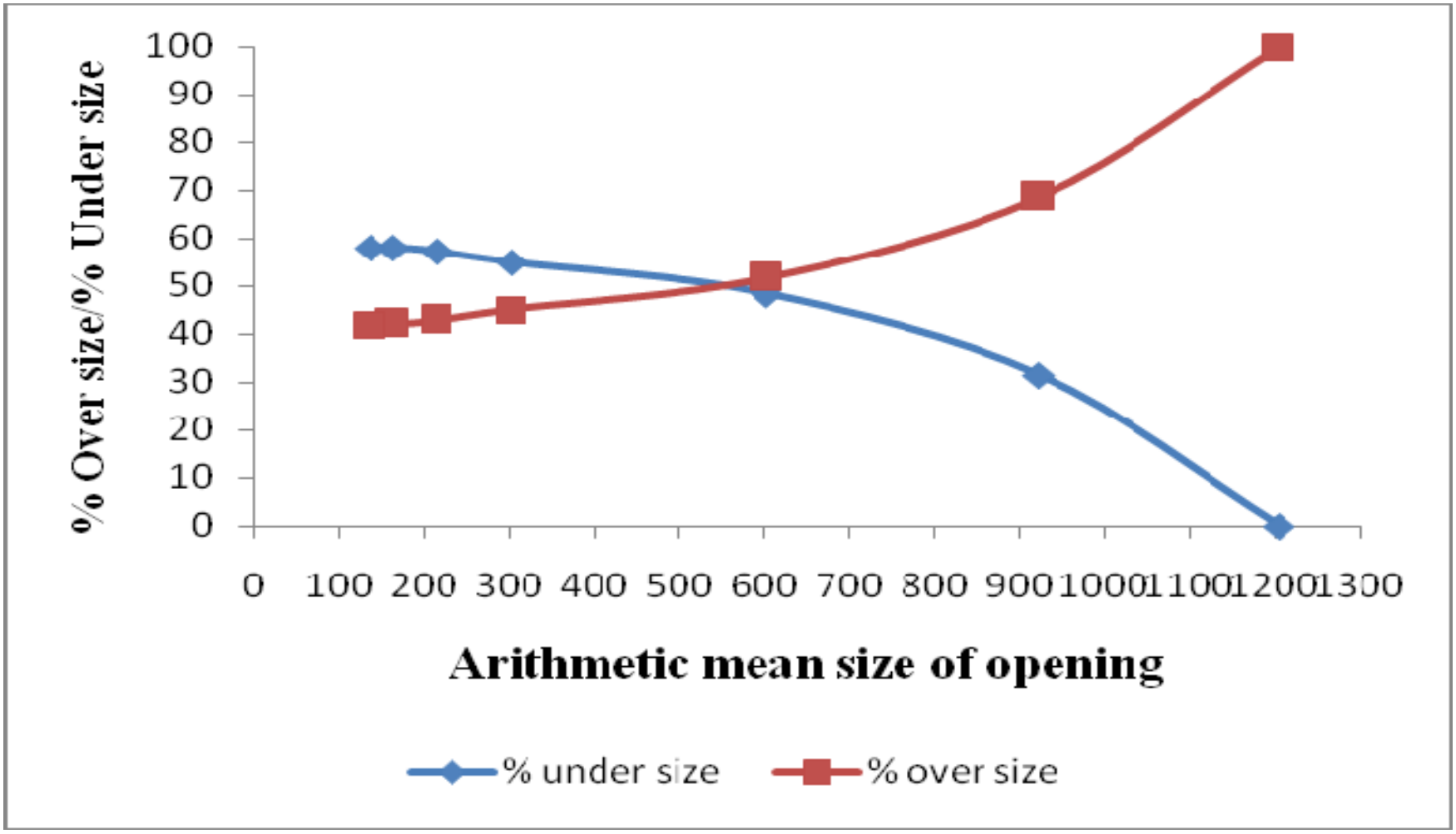



**Figure-16**

**Particle size of F3 formulations by sieving method**

**Table-27**

**Particle size of F4 formulations by sieving method**

| Sieve number | Aperture sieve size as per IP specification (µm) | Arithmetic mean size of opening (d) µm | Weight of granules retained on a sieve (over size) gm | % weight of granules retained (under size) (n) | Cumulative % of granules retained (under size) | Weight size (n x d) | % under size | % under size | % over size (100 - % under size) |
|---|---|---|---|---|---|---|---|---|---|
| 10 | 1700 | 1205 | 0 | 0 | 0 | 0 | 0 | 0 | 100 |
| 22 | 710 | 922.5 | 5.68 | 56.76 | 56.76 | 52361.1 | 52361.1 | 52.3611 | 47.6389 |
| 36 | 425 | 602.5 | 2.58 | 25.84 | 82.6 | 15568.6 | 67929.7 | 67.9297 | 32.0703 |
| 44 | 355 | 302.5 | 1.29 | 12.87 | 95.47 | 3893.175 | 71822.875 | 71.822875 | 28.177125 |
| 60 | 250 | 215 | 0.33 | 3.31 | 98.78 | 711.65 | 72534.525 | 72.534525 | 27.465475 |
| 85 | 180 | 165 | 0.07 | 0.72 | 99.5 | 118.8 | 72653.325 | 72.653325 | 27.346675 |
| 100 | 150 | 137.5 | 0.05 | 0.5 | 100 | 68.75 | 72722.075 | 72.722075 | 27.277925 |
| 120 | 125 | 62.5 | 0 | 0 | 100 | 0 | 72722.075 | 72.722075 | 27.277925 |
| Pan | 0 | 0 | | 0 | 100 | 0 | 72722.075 | 72.722075 | 27.277925 |
| | | | 10 | ∑n= 100 | | ∑(nxd) =72722.075 | | | |

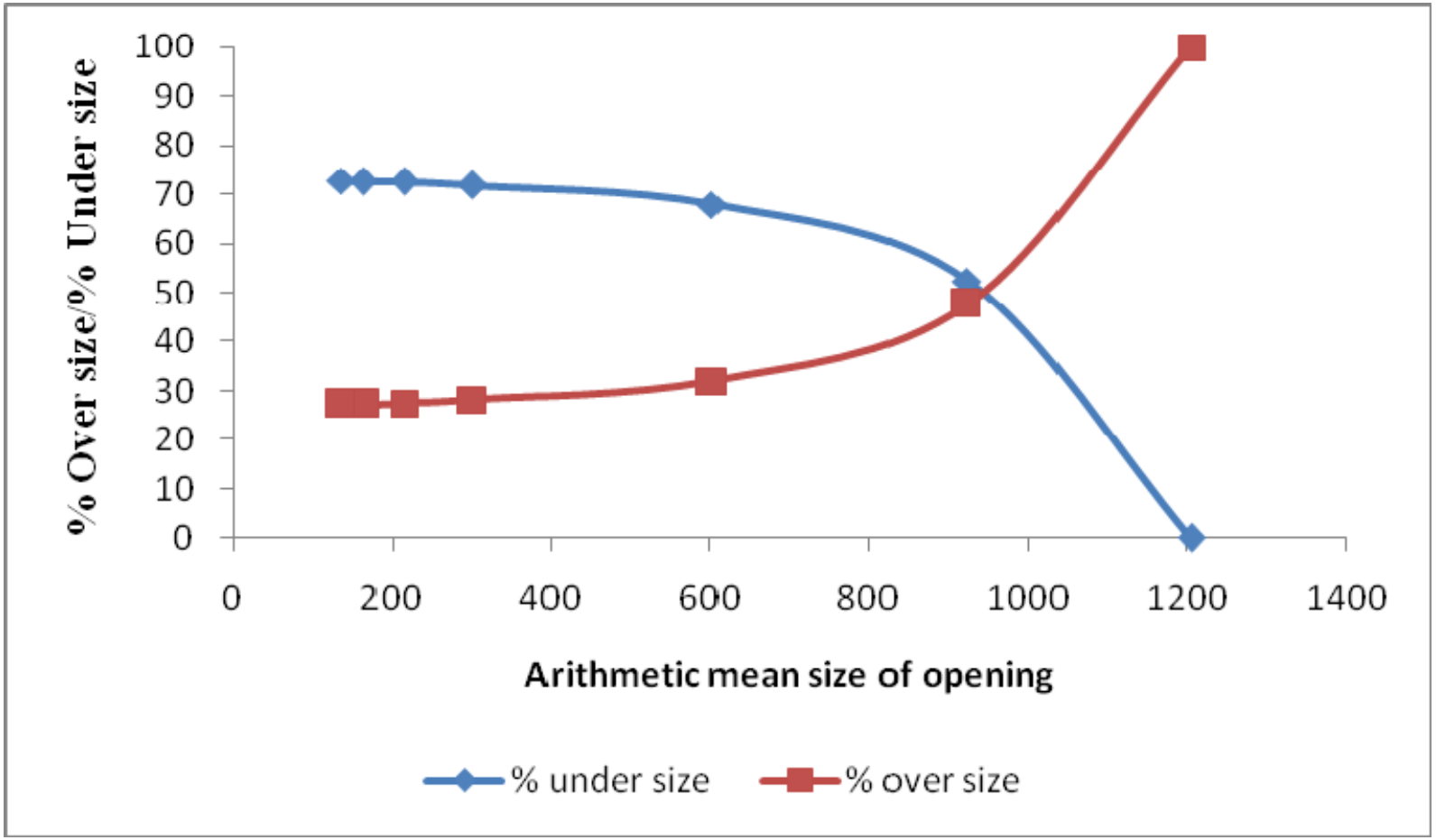



**Figure-17**

**Particle size of F4 formulations by sieving method**

**Table-28**

**Particle size of F5 formulations by sieving method**

| Sieve number | Aperture sieve size as per IP specification (µm) | Arithmetic mean size of opening (d) µm | Weight of granules retained on a sieve (over size) gm | % weight of granules retained (under size) (n) | Cumulative % of granules retained (under size) | Weight size (n x d) | % under size | % under size | % over size (100 - % under size) |
|---|---|---|---|---|---|---|---|---|---|
| 10 | 1700 | 1205 | 0 | 0 | 0 | 0 | 0 | 0 | 100 |
| 22 | 710 | 922.5 | 5.34 | 53.4 | 53.4 | 49261.5 | 49261.5 | 49.2615 | 50.7385 |
| 36 | 425 | 602.5 | 3.1 | 31 | 84.4 | 18677.5 | 67939 | 67.939 | 32.061 |
| 44 | 355 | 302.5 | 1.21 | 12.1 | 96.5 | 3660.25 | 71599.25 | 71.59925 | 28.40075 |
| 60 | 250 | 215 | 0.26 | 2.6 | 99.1 | 559 | 72158.25 | 72.15825 | 27.84175 |
| 85 | 180 | 165 | 0.09 | 0.9 | 100 | 148.5 | 72306.75 | 72.30675 | 27.69325 |
| 100 | 150 | 137.5 | 0 | 0 | 100 | 0 | 72306.75 | 72.30675 | 27.69325 |
| 120 | 125 | 62.5 | 0 | 0 | 100 | 0 | 72306.75 | 72.30675 | 27.69325 |
| Pan | 0 | 0 | | 0 | 100 | 0 | 72306.75 | 72.30675 | 27.69325 |
| | | | 10 | ∑n= 100 | | ∑(nxd) =72306.75 | | | |

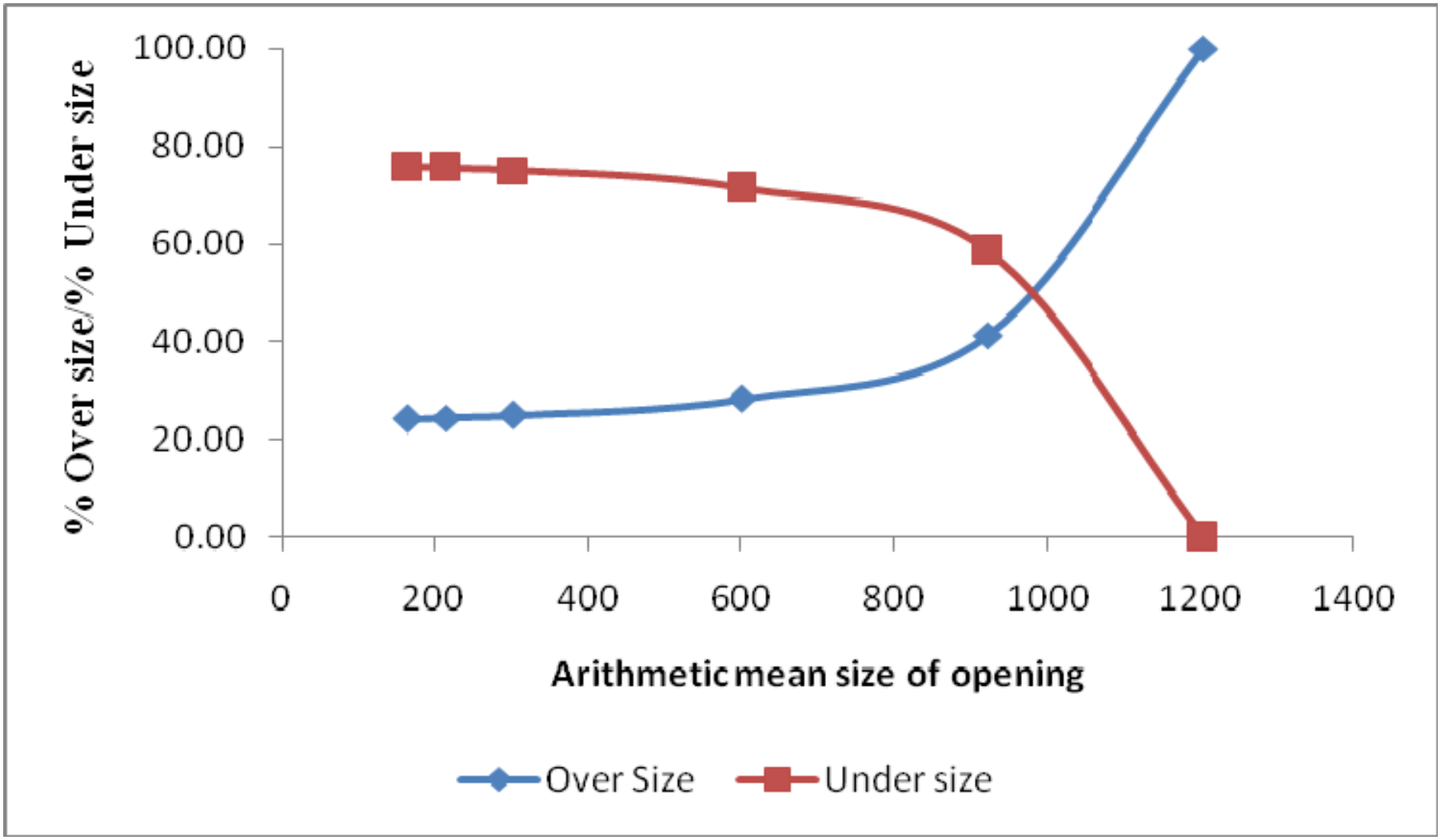



**Figure-18**

**Particle size of F5 formulations by sieving method**

**Table-29**

**Particle size of F6 formulations by sieving method**

| Sieve number | Aperture sieve size as per IP specification (µm) | Arithmetic mean size of opening (d) µm | Weight of granules retained on a sieve (over size) gm | % weight of granules retained (under size) (n) | Cumulative % of granules retained (under size) | Weight size (n x d) | % under size | % under size | % over size (100 - % under size) |
|---|---|---|---|---|---|---|---|---|---|
| 10 | 1700 | 1205 | 0 | 0 | 0 | 0 | 0 | 0 | 100 |
| 22 | 710 | 922.5 | 5.95 | 59.5 | 59.5 | 54888.75 | 54888.8 | 54.8888 | 45.1113 |
| 36 | 425 | 602.5 | 2.3 | 23 | 82.5 | 13857.5 | 68746.3 | 68.7463 | 31.2538 |
| 44 | 355 | 302.5 | 1.2 | 12 | 94.5 | 3630 | 72376.3 | 72.3763 | 27.6238 |
| 60 | 250 | 215 | 0.3 | 3 | 97.5 | 645 | 73021.3 | 73.0213 | 26.9788 |
| 85 | 180 | 165 | 0.18 | 1.8 | 99.3 | 297 | 73318.3 | 73.3183 | 26.6818 |
| 100 | 150 | 137.5 | 0.07 | 3.04348 | 102.343 | 418.478261 | 73736.7 | 73.7367 | 26.2633 |
| 120 | 125 | 62.5 | 0 | 0 | 102.343 | 0 | 73736.7 | 73.7367 | 26.2633 |
| Pan | 0 | 0 | | 0 | 102.343 | 0 | 73736.7 | 73.7367 | 26.2633 |
| | | | 10 | ∑n= 100 | | ∑(nxd) =73736.7283 | | | |

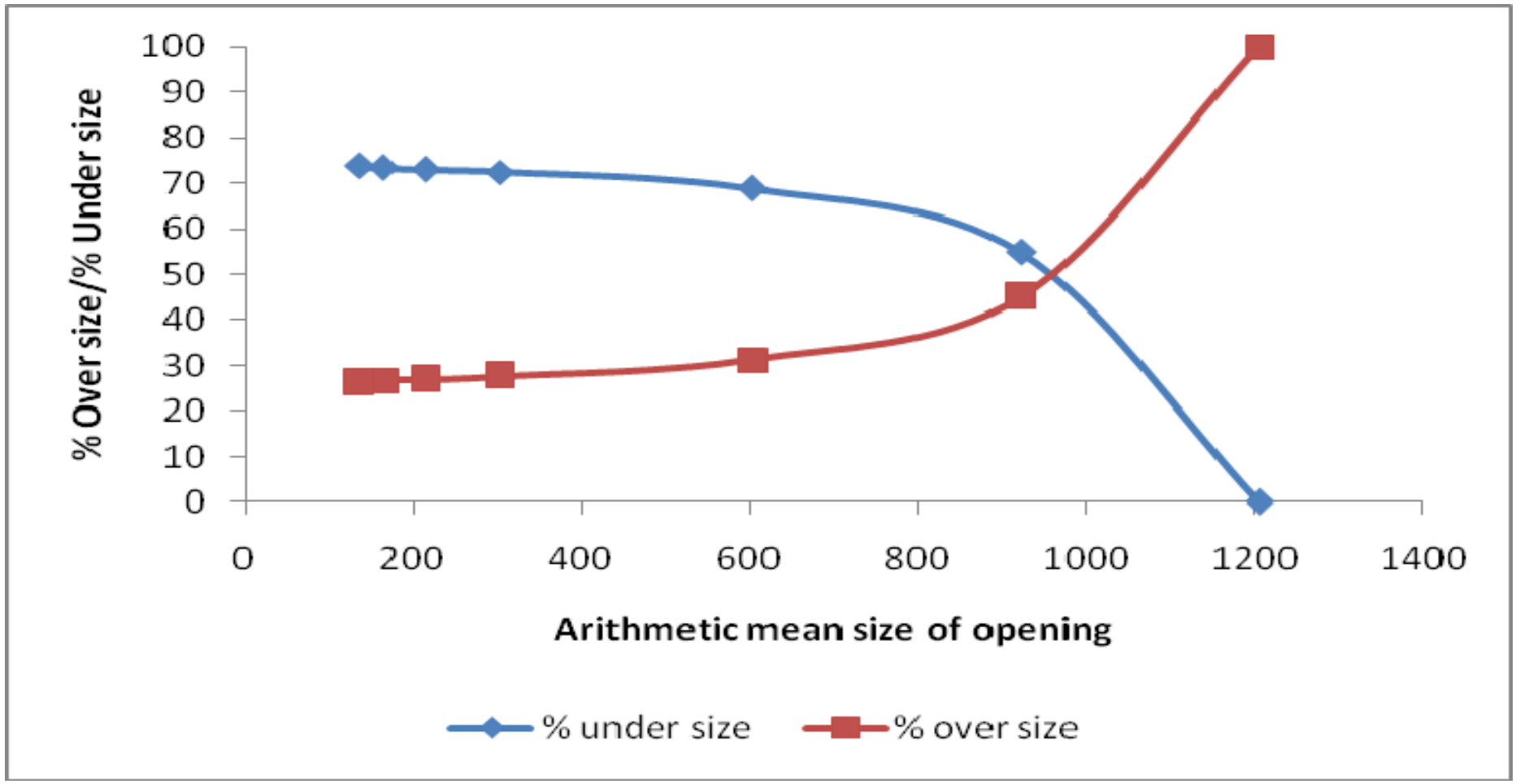



**Figure-19**

**Particle size of F6 formulations by sieving method**

**Table-30**

**Consolidated data for particle size determinations by sieving method**

| | **Formulations** | **Particle size (µm)** |
|---|---|---|
| Without chitosan | F1 | 584.34 |
| | F2 | 583.95 |
| | F3 | 591.19 |
| With chitosan | F4 | 727.22 |
| | F5 | 723.07 |
| | F6 | 737.37 |

**Corollary:**

The formulations prepared without chitosan showed the particle size of 584.34 µm for F1 formulations, 583.95 µm for F2 formulations and 591.19 µm for F3 formulations. The formulations prepared with chitosan showed the particle size of 727.22 µm for F4 formulations, 723.07 µm for F5 formulations and 737.37 µm for F6 formulations. The results of the microparticle studies showed the formulations prepared without chitosan had decreased in particle size when compared to the formulations prepared with chitosan.

**In-Vitro Drug Release Studies**

The release of pure diclofenac sodium, sulfamethoxazole individually and also by combining both drugs and the prepared microparticle formulations were determined in lab India disso apparatus at 37±2°C for 30 min. stirring speed of 50 rpm in pH 7.4. The percentage drug released at different time intervals were noted and the results were tabulated in table-31 to 46 and figure-20 to 23

**Table-31**

***In-Vitro* drug release studies of pure Diclofenac sodium**

| S. No. | Time (min.) | Absorbance at 276 nm | Concentration (µg/ml) | Drug release (mg) | % Drug release |
|---|---|---|---|---|---|
| 1 | 5 | 0.039 | 0.882352941 | 7.941176471 | 15.88235294 |
| 2 | 10 | 0.051 | 1.235294118 | 11.11764706 | 22.23529412 |
| 3 | 15 | 0.069 | 1.764705882 | 15.88235294 | 31.76470588 |
| 4 | 20 | 0.081 | 2.117647059 | 19.05882353 | 38.11764706 |
| 5 | 25 | 0.092 | 2.441176471 | 21.97058824 | 43.94117647 |
| 6 | 30 | 0.096 | 2.558823529 | 23.02941176 | 46.05882353 |

**Table-32**

***In-Vitro* drug release studies of pure Sulfamethoxazole**

| S. No. | Time (min.) | Absorbance at 257 nm | Concentration (µg/ml) | Drug release (mg) | % Drug release |
|---|---|---|---|---|---|
| 1 | 5 | 0.138 | 2.03125 | 18.28125 | 18.28125 |
| 2 | 10 | 0.169 | 2.515625 | 22.640625 | 22.640625 |
| 3 | 15 | 0.197 | 2.953125 | 26.578125 | 26.578125 |
| 4 | 20 | 0.228 | 3.4375 | 30.9375 | 30.9375 |
| 5 | 25 | 0.269 | 4.078125 | 36.703125 | 36.703125 |
| 6 | 30 | 0.291 | 4.421875 | 39.796875 | 39.796875 |

**Table-33**

***In-Vitro* Drug release studies of pure diclofenac sodium in combination with sulfamethoxazole**

| S. No. | Time (min.) | Absorbance at 276 nm | Concentration (µg/ml) | Drug release (mg) | % Drug release |
|---|---|---|---|---|---|
| 1 | 5 | 0.061 | 0.935483871 | 8.419354839 | 16.83870968 |
| 2 | 10 | 0.089 | 1.387096774 | 12.48387097 | 24.96774194 |
| 3 | 15 | 0.119 | 1.870967742 | 16.83870968 | 33.67741935 |
| 4 | 20 | 0.153 | 2.419354839 | 21.77419355 | 43.5483871 |
| 5 | 25 | 0.18 | 2.85483871 | 25.69354839 | 51.38709677 |
| 6 | 30 | 0.21 | 3.338709677 | 30.0483871 | 60.09677419 |

**Table-34**

***In-Vitro* drug release studies of pure sulfamethoxazole in combination with diclofenac sodium**

| S. No. | Time (min.) | Absorbance at 257 nm | Concentration (µg/ml) | Drug release (mg) | % Drug release |
|---|---|---|---|---|---|
| 1 | 5 | 0.313 | 3.522727273 | 31.70454545 | 31.70454545 |
| 2 | 10 | 0.389 | 4.386363636 | 39.47727273 | 39.47727273 |
| 3 | 15 | 0.412 | 4.647727273 | 41.82954545 | 41.82954545 |
| 4 | 20 | 0.476 | 5.375 | 48.375 | 48.375 |
| 5 | 25 | 0.512 | 5.784090909 | 52.05681818 | 52.05681818 |
| 6 | 30 | 0.581 | 6.568181818 | 59.11363636 | 59.11363636 |

**Table-35**

***In-Vitro* drug release studies of F1 formulation**

| S. No. | Time (min.) | Absorbance at 276 nm | Concentration (µg/ml) | Drug release (mg) | % Drug release |
|---|---|---|---|---|---|
| 1 | 5 | 0.012 | 0.088235294 | 0.794117647 | 15.88235294 |
| 2 | 10 | 0.014 | 0.147058824 | 1.323529412 | 26.47058824 |
| 3 | 15 | 0.016 | 0.205882353 | 1.852941176 | 37.05882353 |
| 4 | 20 | 0.018 | 0.264705882 | 2.382352941 | 47.64705882 |
| 5 | 25 | 0.021 | 0.352941176 | 3.176470588 | 63.52941176 |
| 6 | 30 | 0.022 | 0.382352941 | 3.441176471 | 68.82352941 |

**Table-36**

***In-Vitro* drug release studies of F2 formulation**

| S. No. | Time (min.) | Absorbance at 257 nm | Concentration (µg/ml) | Drug release (mg) | % Drug release |
|---|---|---|---|---|---|
| 1 | 5 | 0.03 | 0.34375 | 3.09375 | 30.9375 |
| 2 | 10 | 0.038 | 0.46875 | 4.21875 | 42.1875 |
| 3 | 15 | 0.038 | 0.46875 | 4.21875 | 42.1875 |
| 4 | 20 | 0.053 | 0.703125 | 6.328125 | 63.28125 |
| 5 | 25 | 0.054 | 0.71875 | 6.46875 | 64.6875 |
| 6 | 30 | 0.056 | 0.75 | 6.75 | 67.5 |

**Table-37**

***In-Vitro* drug release studies of Diclofenac sodium in F3 formulation**

| S. No. | Time (min.) | Absorbance at 276 nm | Concentration (µg/ml) | Drug release (mg) | % Drug release |
|---|---|---|---|---|---|
| 1 | 5 | 0.009 | 0.096774194 | 0.870967742 | 17.41935484 |
| 2 | 10 | 0.012 | 0.14516129 | 1.306451613 | 26.12903226 |
| 3 | 15 | 0.017 | 0.225806452 | 2.032258065 | 40.64516129 |
| 4 | 20 | 0.021 | 0.290322581 | 2.612903226 | 52.25806452 |
| 5 | 25 | 0.026 | 0.370967742 | 3.338709677 | 66.77419355 |
| 6 | 30 | 0.029 | 0.419354839 | 3.774193548 | 75.48387097 |

**Table-38**

***In-Vitro* drug release studies of Sulfamethoxazole in F3 formulation**

| S. No. | Time (min.) | Absorbance at 257 nm | Concentration (µg/ml) | Drug release (mg) | % Drug release |
|---|---|---|---|---|---|
| 1 | 5 | 0.047 | 0.5 | 4.5 | 45 |
| 2 | 10 | 0.052 | 0.556818182 | 5.011363636 | 50.11363636 |
| 3 | 15 | 0.058 | 0.625 | 5.625 | 56.25 |
| 4 | 20 | 0.059 | 0.636363636 | 5.727272727 | 57.27272727 |
| 5 | 25 | 0.074 | 0.806818182 | 7.261363636 | 72.61363636 |
| 6 | 30 | 0.083 | 0.909090909 | 8.181818182 | 81.81818182 |

**Table-39**

***In-Vitro* drug release studies of F4 formulation**

| S. No. | Time (min.) | Absorbance at 276 nm | Concentration (µg/ml) | Drug release (mg) | % Drug release |
|---|---|---|---|---|---|
| 1 | 5 | 0.013 | 0.117647059 | 1.058823529 | 21.17647059 |
| 2 | 10 | 0.016 | 0.205882353 | 1.852941176 | 37.05882353 |
| 3 | 15 | 0.02 | 0.323529412 | 2.911764706 | 58.23529412 |
| 4 | 20 | 0.022 | 0.382352941 | 3.441176471 | 68.82352941 |
| 5 | 25 | 0.023 | 0.411764706 | 3.705882353 | 74.11764706 |
| 6 | 30 | 0.025 | 0.470588235 | 4.235294118 | 84.70588235 |

**Table-40**

***In-Vitro* drug release studies of F5 formulation**

| S. No. | Time (min.) | Absorbance at 257 nm | Concentration (µg/ml) | Drug release (mg) | % Drug release |
|---|---|---|---|---|---|
| 1 | 5 | 0.049 | 0.640625 | 5.765625 | 57.65625 |
| 2 | 10 | 0.049 | 0.640625 | 5.765625 | 57.65625 |
| 3 | 15 | 0.056 | 0.75 | 6.75 | 67.5 |
| 4 | 20 | 0.062 | 0.84375 | 7.59375 | 75.9375 |
| 5 | 25 | 0.065 | 0.890625 | 8.015625 | 80.15625 |
| 6 | 30 | 0.067 | 0.921875 | 8.296875 | 82.96875 |

**Table-41**

***In-Vitro* drug release studies of Diclofenac sodium in F6 formulation**

| S. No. | Time (min.) | Absorbance at 276 nm | Concentration (µg/ml) | Drug release (mg) | % Drug release |
|---|---|---|---|---|---|
| 1 | 5 | 0.014 | 0.177419355 | 1.596774194 | 31.9355 |
| 2 | 10 | 0.017 | 0.225806452 | 2.032258065 | 40.6452 |
| 3 | 15 | 0.021 | 0.290322581 | 2.612903226 | 52.2581 |
| 4 | 20 | 0.027 | 0.387096774 | 3.483870968 | 69.6774 |
| 5 | 25 | 0.032 | 0.467741935 | 4.209677419 | 84.1935 |
| 6 | 30 | 0.037 | 0.548387097 | 4.935483871 | 98.7097 |

**Table-42**

***In-Vitro* drug release studies of Sulfamethoxazole in F6 formulation**

| S. No. | Time (min.) | Absorbance at 257 nm | Concentration (µg/ml) | Drug release (mg) | % Drug release |
|---|---|---|---|---|---|
| 1 | 5 | 0.055 | 0.590909091 | 5.318181818 | 53.18181818 |
| 2 | 10 | 0.071 | 0.772727273 | 6.954545455 | 69.54545455 |
| 3 | 15 | 0.073 | 0.795454545 | 7.159090909 | 71.59090909 |
| 4 | 20 | 0.083 | 0.909090909 | 8.181818182 | 81.81818182 |
| 5 | 25 | 0.091 | 1 | 9 | 90 |
| 6 | 30 | 0.1 | 1.102272727 | 9.920454545 | 99.20454545 |

**Table-43**

**Comparison of *In-Vitro* drug release studies of pure Diclofenac sodium, F1 and F4 formulation**

| S. No. | Time (min.) | % Drug released | | |
|---|---|---|---|---|
| | | Pure Diclofenac sodium | Formulations | |
| | | | F1 | F4 |
| 1 | 5 | 15.88 | 15.88 | 21.18 |
| 2 | 10 | 22.24 | 26.47 | 37.06 |
| 3 | 15 | 31.76 | 37.06 | 58.24 |
| 4 | 20 | 38.12 | 47.65 | 68.82 |
| 5 | 25 | 43.94 | 63.53 | 74.12 |
| 6 | 30 | 46.06 | 68.82 | 84.71 |

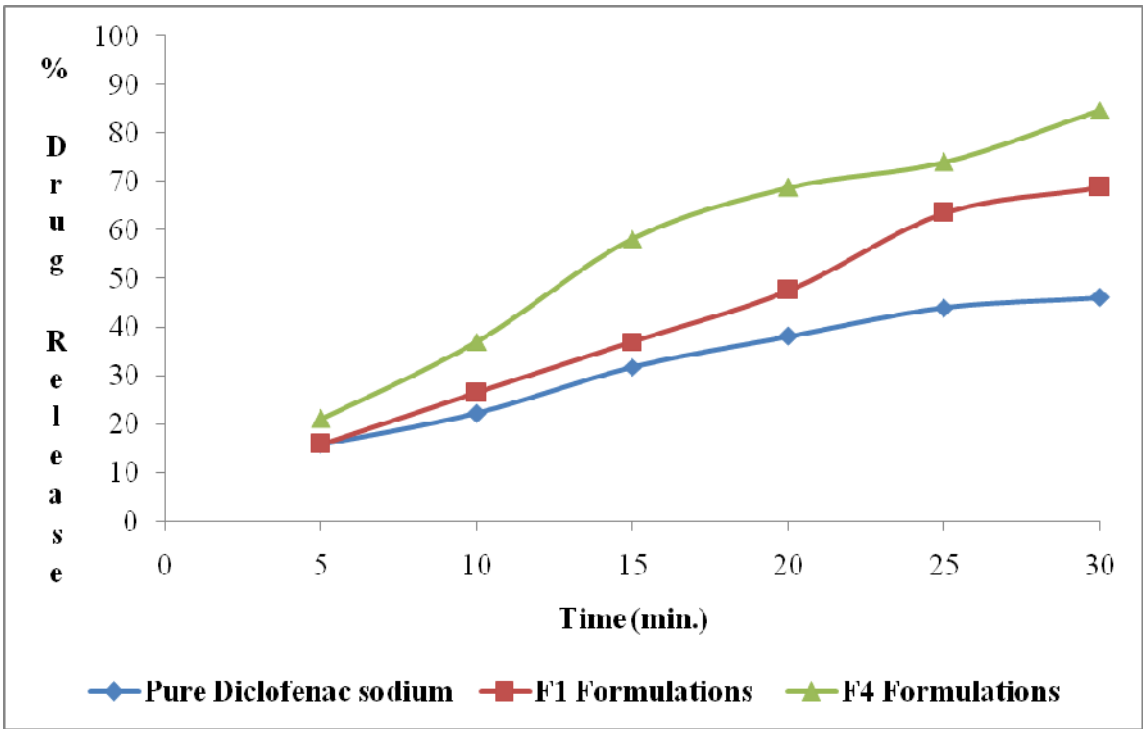



**Figure-20**

**Comparison of *In-Vitro* drug release studies of pure Diclofenac sodium, F1 and F4 formulation**

**Table-44**

**Comparison of *In-Vitro* drug release studies of pure Sulfamethoxazole, F2 and F5 formulation**

| S. No. | Time (min.) | % Drug released | | |
|---|---|---|---|---|
| | | Pure Sulfamethoxazole | Formulations | |
| | | | F2 | F5 |
| 1 | 5 | 18.28 | 30.94 | 57.66 |
| 2 | 10 | 22.64 | 42.19 | 57.66 |
| 3 | 15 | 26.58 | 42.19 | 67.50 |
| 4 | 20 | 30.94 | 63.28 | 75.94 |
| 5 | 25 | 36.70 | 64.69 | 80.16 |
| 6 | 30 | 39.80 | 67.50 | 82.97 |

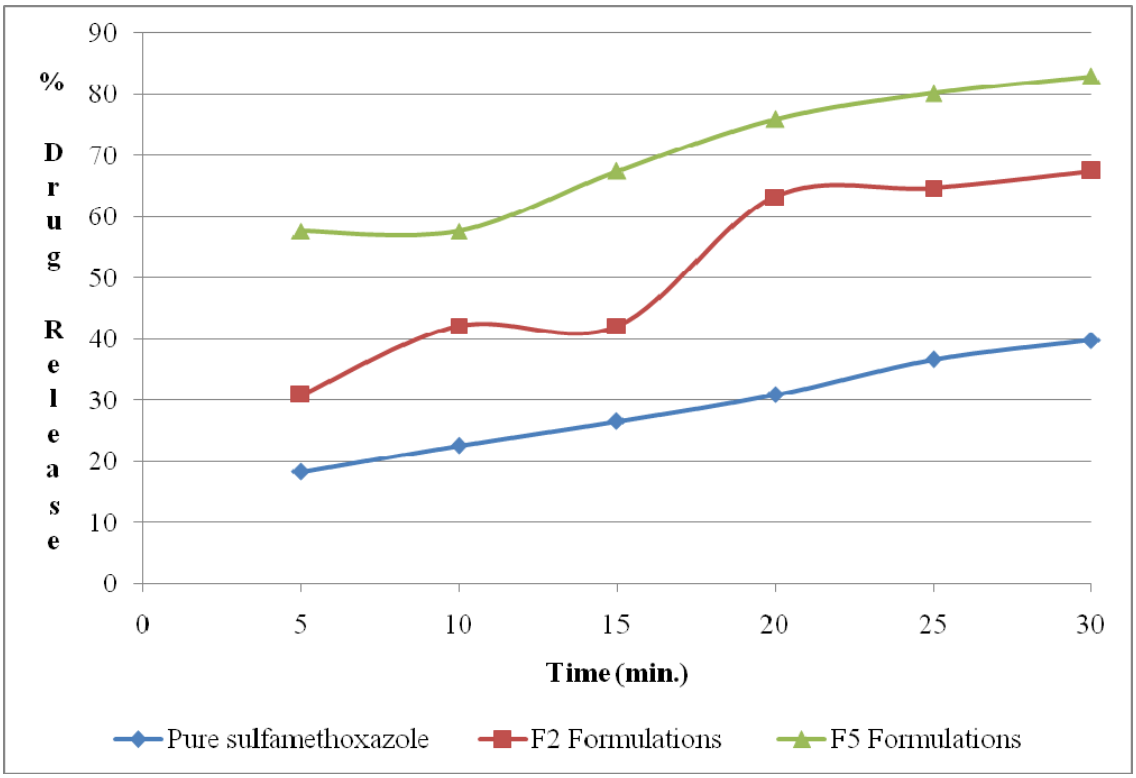



**Figure-21**

**Comparison of *In-Vitro* drug release studies of pure Sulfamethoxazole, F2 and F5 formulation**

**Table-45**

**Comparison of *In-Vitro* drug release studies of pure Diclofenac sodium in combination with Sulfamethoxazole, F3 and F6 formulation**

| S. No. | Time (min.) | % Drug released | | |
|---|---|---|---|---|
| | | Pure Diclofenac sodium in combination with pure Sulfamethoxazole | Diclofenac sodium in F3 Formulations | Diclofenac sodium in F6 Formulations |
| 1 | 5 | 16.84 | 17.42 | 31.94 |
| 2 | 10 | 24.97 | 26.13 | 40.65 |
| 3 | 15 | 33.68 | 40.65 | 52.26 |
| 4 | 20 | 43.55 | 52.26 | 69.68 |
| 5 | 25 | 51.39 | 66.77 | 84.19 |
| 6 | 30 | 60.10 | 75.48 | 98.71 |

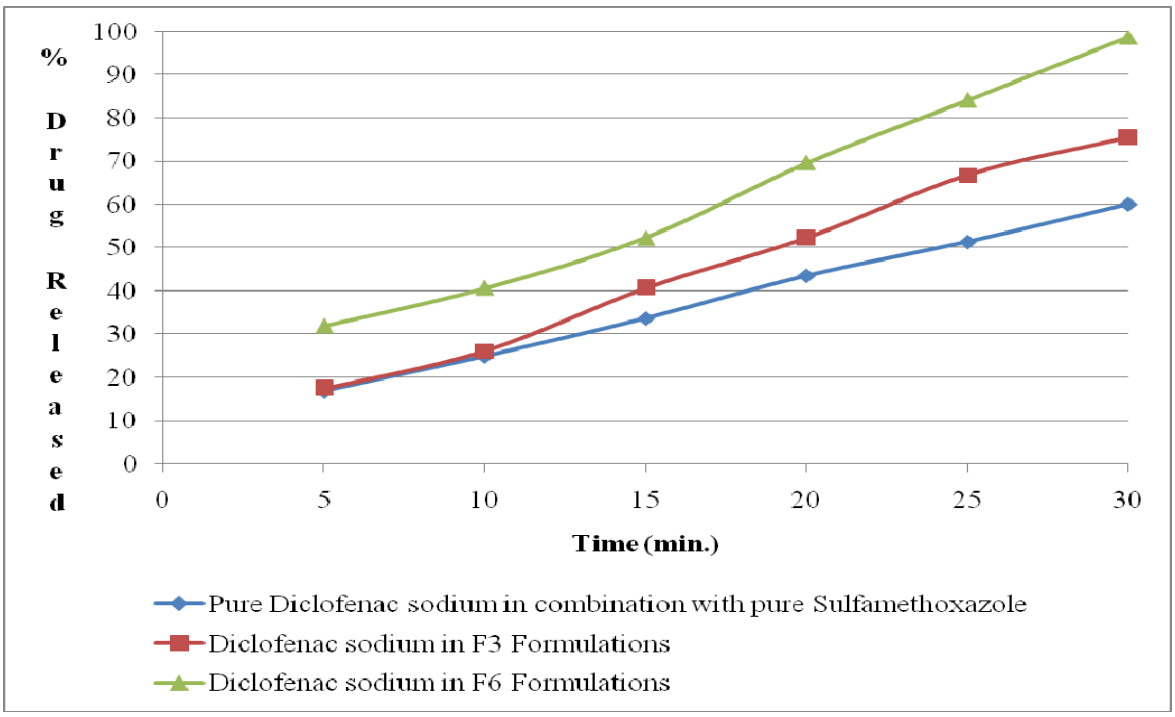



**Figure-22**

**Comparison of *In-Vitro* drug release studies of pure Diclofenac sodium in combination with Sulfamethoxazole, F3 and F6 formulation**

**Table-46**

**Comparison of *In-Vitro* drug release studies of pure Sulfamethoxazole in combination with Diclofenac sodium, F3 and F6 formulation**

| S. No. | Time (min.) | % Drug released | | |
|---|---|---|---|---|
| | | Pure Sulfamethoxazole in combination with pure Diclofenac sodium | Sulfamethoxazole in F3 Formulations | Sulfamethoxazole in F6 Formulations |
| 1 | 5 | 31.70 | 45.00 | 53.18 |
| 2 | 10 | 39.48 | 50.11 | 69.55 |
| 3 | 15 | 41.83 | 56.25 | 71.59 |
| 4 | 20 | 48.38 | 57.27 | 81.82 |
| 5 | 25 | 52.06 | 72.61 | 90.00 |
| 6 | 30 | 59.11 | 81.82 | 99.20 |

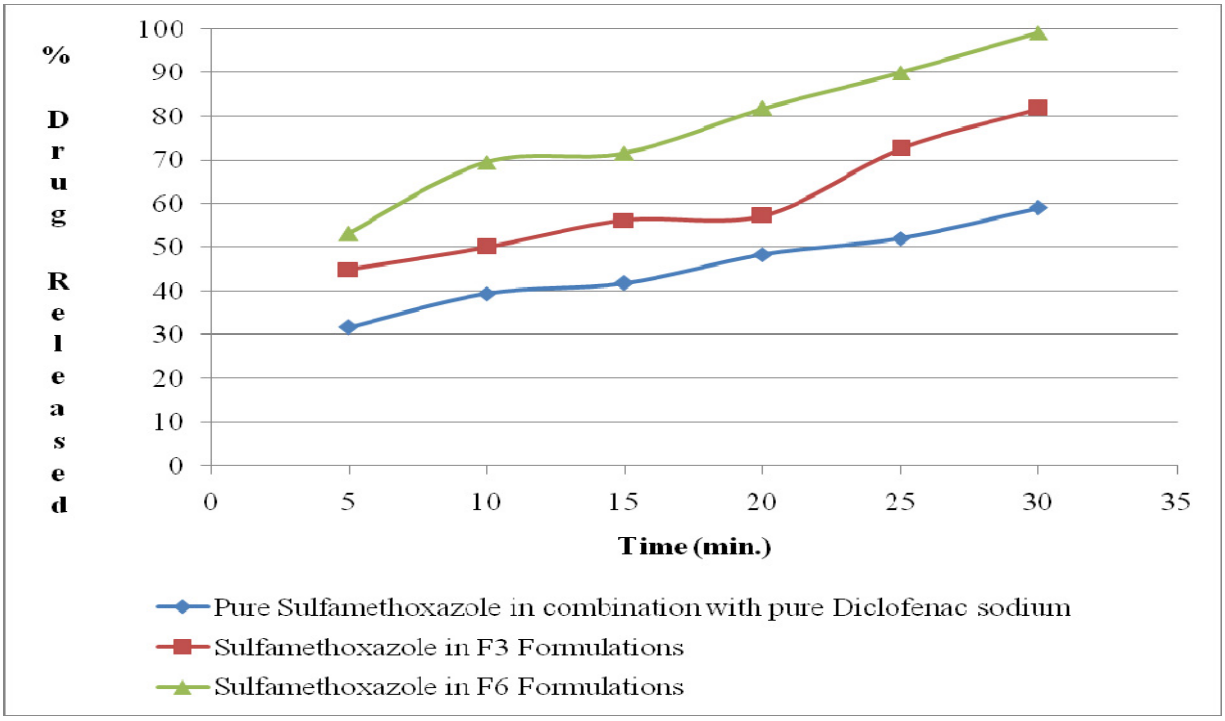



**Figure-23**

**Comparison of *In-Vitro* drug release studies of pure Sulfamethoxazole in combination with Diclofenac sodium, F3 and F6 formulation**

**Corollary:**

At 30 min. 46.06% of drug was released in pure Diclofenac sodium, 39.8% of drug was released in pure Sulfamethoxazole and 60.10% of Diclofenac sodium was released in combination with Sulfamethoxazole, similarly 59.11 % of Sulfamethoxazole was released in combination with Diclofenac sodium. The drug release for both the pure drug Diclofenac sodium and Sulfamethoxazole in combination showed increased in drug release when compared to individual release.

The Diclofenac sodium prepared without chitosan (F1) showed 68.82% of drug release at 30 min. The Diclofenac sodium prepared without chitosan (F3) in combination with Sulfamethoxazole 75.48% of drug release at 30 min. The Diclofenac sodium prepared with chitosan (F4) showed 84.71% of drug release at 30 min. The Diclofenac sodium prepared with chitosan (F6) in combination with Sulfamethoxazole showed 98.71% of drug release at 30 min.

The Sulfamethoxazole prepared without chitosan (F2) showed 67.5% of drug release at 30 min. The sulfamethoxazole prepared without chitosan (F3) in combination with Diclofenac sodium showed 81.82% of drug release at 30 min. The Sulfamethoxazole prepared with chitosan (F5) showed 82.97% of drug release at 30 min. The sulfamethoxazole prepared with chitosan (F6) in combination with Diclofenac sodium showed 99.21% of drug release at 30 min.

The drug released study results showed that the release of drugs from microparticles is more in formulations containing chitosan. Chitosan may increase the release of the drug.

**Gel evaluation studies:**

The prepared gels of different concentrations were evaluated for spreadability and consistency test.

**Spreadability and consistency test:**

Different concentrations of gel were prepared by carbomer 940. The spreadability and consistency test were performed for different gel concentrations (0.05% to 1.25% w/v) to determine the spreading tendency and consistency of the gel. The results were shown in **Table No.47 and 48**

**Table-47**

**Spreadability test for different gel concentrations**

| Concentrations (%w/v) | M (gm) | L (cm) | T (Sec) | S = ML/T | Average |
|---|---|---|---|---|---|
| 0.50% | 6 | 7.5 | 3 | 15 | 15.20±0.07959 |
| | 6 | 7.5 | 2.8 | 16.07 | |
| | 6 | 7.5 | 3.1 | 14.52 | |
| 0.75% | 6 | 7.5 | 7 | 6.43 | 6.59 ±0.1463 |
| | 6 | 7.5 | 6.8 | 6.62 | |
| | 6 | 7.5 | 6.7 | 6.72 | |
| 1.00% | 6 | 7.5 | 11.9 | 3.78 | 3.77±0.0481 |
| | 6 | 7.5 | 11.8 | 3.81 | |
| | 6 | 7.5 | 12.1 | 3.72 | |
| 1.25% | 6 | 7.5 | 16.8 | 2.68 | 2.63±0.0537 |
| | 6 | 7.5 | 17.1 | 2.63 | |
| | 6 | 7.5 | 17.5 | 2.57 | |
| 1.5% | 6 | 7.5 | 25.1 | 1.79 | 1.75±0.0442 |
| | 6 | 7.5 | 26.4 | 1.7 | |
| | 6 | 7.5 | 25.8 | 1.74 | |

**Table-48**

**Consistency test for different gel concentrations**

| Concentrations (%w/v) | Taken gel (gm) in tube | Applied weight over the tube | Average |
|---|---|---|---|
| 0.50% | | 125 | 126.667±1.5275 |
| | | 127 | |
| | | 128 | |
| 0.75% | | 150 | 150.667±1.1547 |
| | | 150 | |
| | | 152 | |
| 1.00% | | 184 | 184.667±0.5774 |
| | | 185 | |
| | | 185 | |
| 1.25% | | 220 | 221.667±2.8868 |
| | | 220 | |
| | | 225 | |
| 1.5% | | 295 | 295±0 |
| | | 295 | |
| | 10 | 295 | |

**Gel Permeation Studies**

Two gel concentrations were selected (0.75%w/v and 1% w/v) based on the results of spreadability and consistency test. The microparticles were mixed with 0.75%w/v and 1% w/v concentrations and the permeation studies were performed. The results of the permeation studies were shown in table-49 to 66 and figure- 24 and 25.

**Table-49**

**Release of F1 formulation microparticles from 0.75 % w/v carbomer (940) gel**

| S. No. | Time (min.) | Absorbance at 276 nm | Concentration (µg/ml) | Drug released (mg) | % Drug released |
|---|---|---|---|---|---|
| 1 | 5 | 0.001 | 0.02941176 | 0.029411765 | 0.58823529 |
| 2 | 10 | 0.002 | 0.05882353 | 0.058823529 | 1.17647059 |
| 3 | 15 | 0.003 | 0.08823529 | 0.088235294 | 1.76470588 |
| 4 | 20 | 0.006 | 0.17647059 | 0.176470588 | 3.52941176 |
| 5 | 25 | 0.008 | 0.23529412 | 0.235294118 | 4.70588235 |
| 6 | 30 | 0.009 | 0.26470588 | 0.264705882 | 5.29411765 |
| 7 | 45 | 0.011 | 0.32352941 | 0.323529412 | 6.47058824 |
| 8 | 60 | 0.013 | 0.38235294 | 0.382352941 | 7.64705882 |
| 9 | 90 | 0.015 | 0.44117647 | 0.441176471 | 8.82352941 |
| 10 | 120 | 0.017 | 0.5 | 0.5 | 10 |
| 11 | 150 | 0.019 | 0.55882353 | 0.558823529 | 11.1764706 |
| 12 | 180 | 0.021 | 0.61764706 | 0.617647059 | 12.3529412 |
| 13 | 210 | 0.025 | 0.73529412 | 0.735294118 | 14.7058824 |
| 14 | 240 | 0.028 | 0.82352941 | 0.823529412 | 16.4705882 |
| 15 | 270 | 0.031 | 0.91176471 | 0.911764706 | 18.2352941 |
| 16 | 300 | 0.035 | 1.02941176 | 1.029411765 | 20.5882353 |
| 17 | 330 | 0.04 | 1.17647059 | 1.176470588 | 23.5294118 |
| 18 | 360 | 0.044 | 1.29411765 | 1.294117647 | 25.8823529 |

**Table-50**

**Release of F1 formulation microparticles from 1 % w/v carbomer (940) gel**

| S. No. | Time (min.) | Absorbance at 276 nm | Concentration (µg/ml) | Drug released (mg) | % Drug released |
|---|---|---|---|---|---|
| 1 | 5 | 0 | 0 | 0 | 0 |
| 2 | 10 | 0 | 0 | 0 | 0 |
| 3 | 15 | 0.001 | 0.02941 | 0.02941 | 0.58824 |
| 4 | 20 | 0.001 | 0.02941 | 0.02941 | 0.58824 |
| 5 | 25 | 0.002 | 0.05882 | 0.05882 | 1.17647 |
| 6 | 30 | 0.005 | 0.14706 | 0.14706 | 2.94118 |
| 7 | 45 | 0.007 | 0.20588 | 0.20588 | 4.11765 |
| 8 | 60 | 0.01 | 0.29412 | 0.29412 | 5.88235 |
| 9 | 90 | 0.012 | 0.35294 | 0.35294 | 7.05882 |
| 10 | 120 | 0.014 | 0.41176 | 0.41176 | 8.23529 |
| 11 | 150 | 0.016 | 0.47059 | 0.47059 | 9.41176 |
| 12 | 180 | 0.019 | 0.55882 | 0.55882 | 11.1765 |
| 13 | 210 | 0.022 | 0.64706 | 0.64706 | 12.9412 |
| 14 | 240 | 0.025 | 0.73529 | 0.73529 | 14.7059 |
| 15 | 270 | 0.027 | 0.79412 | 0.79412 | 15.8824 |
| 16 | 300 | 0.03 | 0.88235 | 0.88235 | 17.6471 |
| 17 | 330 | 0.033 | 0.97059 | 0.97059 | 19.4118 |
| 18 | 360 | 0.035 | 1.02941 | 1.02941 | 20.5882 |

**Table-51**

**Release of F2 formulation microparticles from 0.75 % w/v carbomer (940) gel**

| S. No. | Time (min.) | Absorbance at 257 nm | Concentration (µg/ml) | Drug released (mg) | % Drug released |
|---|---|---|---|---|---|
| 1 | 5 | 0.002 | -0.0938 | -0.0938 | -0.9375 |
| 2 | 10 | 0.005 | -0.0469 | -0.0469 | -0.4688 |
| 3 | 15 | 0.009 | 0.01563 | 0.01563 | 0.15625 |
| 4 | 20 | 0.013 | 0.07813 | 0.07813 | 0.78125 |
| 5 | 25 | 0.016 | 0.125 | 0.125 | 1.25 |
| 6 | 30 | 0.02 | 0.1875 | 0.1875 | 1.875 |
| 7 | 45 | 0.025 | 0.26563 | 0.26563 | 2.65625 |
| 8 | 60 | 0.03 | 0.34375 | 0.34375 | 3.4375 |
| 9 | 90 | 0.036 | 0.4375 | 0.4375 | 4.375 |
| 10 | 120 | 0.041 | 0.51563 | 0.51563 | 5.15625 |
| 11 | 150 | 0.046 | 0.59375 | 0.59375 | 5.9375 |
| 12 | 180 | 0.052 | 0.6875 | 0.6875 | 6.875 |
| 13 | 210 | 0.061 | 0.82813 | 0.82813 | 8.28125 |
| 14 | 240 | 0.073 | 1.01563 | 1.01563 | 10.1563 |
| 15 | 270 | 0.085 | 1.20313 | 1.20313 | 12.0313 |
| 16 | 300 | 0.099 | 1.42188 | 1.42188 | 14.2188 |
| 17 | 330 | 0.114 | 1.65625 | 1.65625 | 16.5625 |
| 18 | 360 | 0.128 | 1.875 | 1.875 | 18.75 |

**Table-52**

**Release of F2 formulation microparticles from 1 % w/v carbomer (940) gel**

| S. No. | Time (min.) | Absorbance at 257 nm | Concentration (µg/ml) | Drug released (mg) | % Drug released |
|---|---|---|---|---|---|
| 1 | 5 | 0.001 | -0.1094 | -0.1094 | -1.0938 |
| 2 | 10 | 0.003 | -0.0781 | -0.0781 | -0.7813 |
| 3 | 15 | 0.006 | -0.0313 | -0.0313 | -0.3125 |
| 4 | 20 | 0.01 | 0.03125 | 0.03125 | 0.3125 |
| 5 | 25 | 0.013 | 0.07813 | 0.07813 | 0.78125 |
| 6 | 30 | 0.017 | 0.14063 | 0.14063 | 1.40625 |
| 7 | 45 | 0.02 | 0.1875 | 0.1875 | 1.875 |
| 8 | 60 | 0.024 | 0.25 | 0.25 | 2.5 |
| 9 | 90 | 0.029 | 0.32813 | 0.32813 | 3.28125 |
| 10 | 120 | 0.035 | 0.42188 | 0.42188 | 4.21875 |
| 11 | 150 | 0.04 | 0.5 | 0.5 | 5 |
| 12 | 180 | 0.046 | 0.59375 | 0.59375 | 5.9375 |
| 13 | 210 | 0.053 | 0.70313 | 0.70313 | 7.03125 |
| 14 | 240 | 0.062 | 0.84375 | 0.84375 | 8.4375 |
| 15 | 270 | 0.07 | 0.96875 | 0.96875 | 9.6875 |
| 16 | 300 | 0.079 | 1.10938 | 1.10938 | 11.0938 |
| 17 | 330 | 0.091 | 1.29688 | 1.29688 | 12.9688 |
| 18 | 360 | 0.102 | 1.46875 | 1.46875 | 14.6875 |

**Table-53**

**Release of Diclofenac sodium from F3 formulation microparticles from 0.75 % w/v carbomer (940) gel**

| S. No. | Time (min.) | Absorbance at 276 nm | Concentration (µg/ml) | Drug released (mg) | % Drug released |
|---|---|---|---|---|---|
| 1 | 5 | 0.003 | 0.04839 | 0.04839 | 0.96774 |
| 2 | 10 | 0.005 | 0.08065 | 0.08065 | 1.6129 |
| 3 | 15 | 0.01 | 0.16129 | 0.16129 | 3.22581 |
| 4 | 20 | 0.013 | 0.20968 | 0.20968 | 4.19355 |
| 5 | 25 | 0.016 | 0.25806 | 0.25806 | 5.16129 |
| 6 | 30 | 0.018 | 0.29032 | 0.29032 | 5.80645 |
| 7 | 45 | 0.021 | 0.33871 | 0.33871 | 6.77419 |
| 8 | 60 | 0.025 | 0.40323 | 0.40323 | 8.06452 |
| 9 | 90 | 0.029 | 0.46774 | 0.46774 | 9.35484 |
| 10 | 120 | 0.033 | 0.53226 | 0.53226 | 10.6452 |
| 11 | 150 | 0.04 | 0.64516 | 0.64516 | 12.9032 |
| 12 | 180 | 0.047 | 0.75806 | 0.75806 | 15.1613 |
| 13 | 210 | 0.054 | 0.87097 | 0.87097 | 17.4194 |
| 14 | 240 | 0.06 | 0.96774 | 0.96774 | 19.3548 |
| 15 | 270 | 0.071 | 1.14516 | 1.14516 | 22.9032 |
| 16 | 300 | 0.077 | 1.24194 | 1.24194 | 24.8387 |
| 17 | 330 | 0.085 | 1.37097 | 1.37097 | 27.4194 |
| 18 | 360 | 0.092 | 1.48387 | 1.48387 | 29.6774 |

**Table-54**

**Release of Sulfamethoxazole from F3 formulation microparticles from 0.75 % w/v carbomer (940) gel**

| S. No. | Time (min.) | Absorbance at 257 nm | Concentration (µg/ml) | Drug released (mg) | % Drug released |
|---|---|---|---|---|---|
| 1 | 5 | 0.003 | 0 | 0 | 0 |
| 2 | 10 | 0.006 | 0.05551 | 0.05551 | 0.55507 |
| 3 | 15 | 0.008 | 0.08368 | 0.08368 | 0.83676 |
| 4 | 20 | 0.012 | 0.14001 | 0.14001 | 1.40014 |
| 5 | 25 | 0.016 | 0.19635 | 0.19635 | 1.96352 |
| 6 | 30 | 0.02 | 0.25269 | 0.25269 | 2.5269 |
| 7 | 45 | 0.024 | 0.30903 | 0.30903 | 3.09028 |
| 8 | 60 | 0.029 | 0.37945 | 0.37945 | 3.79451 |
| 9 | 90 | 0.038 | 0.50621 | 0.50621 | 5.06211 |
| 10 | 120 | 0.049 | 0.66114 | 0.66114 | 6.61141 |
| 11 | 150 | 0.057 | 0.77382 | 0.77382 | 7.73817 |
| 12 | 180 | 0.066 | 0.90058 | 0.90058 | 9.00577 |
| 13 | 210 | 0.078 | 1.06959 | 1.06959 | 10.6959 |
| 14 | 240 | 0.091 | 1.25269 | 1.25269 | 12.5269 |
| 15 | 270 | 0.102 | 1.40762 | 1.40762 | 14.0762 |
| 16 | 300 | 0.122 | 1.68931 | 1.68931 | 16.8931 |
| 17 | 330 | 0.142 | 1.971 | 1.971 | 19.71 |
| 18 | 360 | 0.16 | 2.22452 | 2.22452 | 22.2452 |

**Table-55**

**Release of Diclofenac sodium from F3 formulation microparticles from 1 % w/v carbomer (940) gel**

| S. No. | Time (min.) | Absorbance at 276 nm | Concentration (µg/ml) | Drug released (mg) | % Drug released |
|---|---|---|---|---|---|
| 1 | 5 | 0.001 | 0.01613 | 0.01613 | 0.32258 |
| 2 | 10 | 0.003 | 0.04839 | 0.04839 | 0.96774 |
| 3 | 15 | 0.008 | 0.12903 | 0.12903 | 2.58065 |
| 4 | 20 | 0.01 | 0.16129 | 0.16129 | 3.22581 |
| 5 | 25 | 0.012 | 0.19355 | 0.19355 | 3.87097 |
| 6 | 30 | 0.015 | 0.24194 | 0.24194 | 4.83871 |
| 7 | 45 | 0.018 | 0.29032 | 0.29032 | 5.80645 |
| 8 | 60 | 0.02 | 0.32258 | 0.32258 | 6.45161 |
| 9 | 90 | 0.023 | 0.37097 | 0.37097 | 7.41935 |
| 10 | 120 | 0.028 | 0.45161 | 0.45161 | 9.03226 |
| 11 | 150 | 0.034 | 0.54839 | 0.54839 | 10.9677 |
| 12 | 180 | 0.038 | 0.6129 | 0.6129 | 12.2581 |
| 13 | 210 | 0.046 | 0.74194 | 0.74194 | 14.8387 |
| 14 | 240 | 0.053 | 0.85484 | 0.85484 | 17.0968 |
| 15 | 270 | 0.059 | 0.95161 | 0.95161 | 19.0323 |
| 16 | 300 | 0.065 | 1.04839 | 1.04839 | 20.9677 |
| 17 | 330 | 0.069 | 1.1129 | 1.1129 | 22.2581 |
| 18 | 360 | 0.08 | 1.29032 | 1.29032 | 25.8065 |

**Table-56**

**Release of Sulfamethoxazole from F3 formulation microparticles from 1 % w/v carbomer (940) gel**

| S. No. | Time (min.) | Absorbance at 257 nm | Concentration (µg/ml) | Drug released (mg) | % Drug released |
|---|---|---|---|---|---|
| 1 | 5 | 0 | -0.0341 | -0.0341 | -0.3409 |
| 2 | 10 | 0.001 | -0.0227 | -0.0227 | -0.2273 |
| 3 | 15 | 0.004 | 0.01136 | 0.01136 | 0.11364 |
| 4 | 20 | 0.007 | 0.04545 | 0.04545 | 0.45455 |
| 5 | 25 | 0.01 | 0.07955 | 0.07955 | 0.79545 |
| 6 | 30 | 0.014 | 0.125 | 0.125 | 1.25 |
| 7 | 45 | 0.023 | 0.22727 | 0.22727 | 2.27273 |
| 8 | 60 | 0.032 | 0.32955 | 0.32955 | 3.29545 |
| 9 | 90 | 0.044 | 0.46591 | 0.46591 | 4.65909 |
| 10 | 120 | 0.055 | 0.59091 | 0.59091 | 5.90909 |
| 11 | 150 | 0.063 | 0.68182 | 0.68182 | 6.81818 |
| 12 | 180 | 0.072 | 0.78409 | 0.78409 | 7.84091 |
| 13 | 210 | 0.084 | 0.92045 | 0.92045 | 9.20455 |
| 14 | 240 | 0.095 | 1.04545 | 1.04545 | 10.4545 |
| 15 | 270 | 0.108 | 1.19318 | 1.19318 | 11.9318 |
| 16 | 300 | 0.123 | 1.36364 | 1.36364 | 13.6364 |
| 17 | 330 | 0.136 | 1.51136 | 1.51136 | 15.1136 |
| 18 | 360 | 0.158 | 1.76136 | 1.76136 | 17.6136 |

**Table-57**

**Release of F4 formulation microparticles from 0.75 % w/v carbomer (940) gel**

| S. No. | Time (min.) | Absorbance at 276 nm | Concentration (µg/ml) | Drug released (mg) | % Drug released |
|---|---|---|---|---|---|
| 1 | 5 | 0.002 | 0.05882353 | 0.058823529 | 1.176470588 |
| 2 | 10 | 0.003 | 0.08823529 | 0.088235294 | 1.764705882 |
| 3 | 15 | 0.006 | 0.17647059 | 0.176470588 | 3.529411765 |
| 4 | 20 | 0.008 | 0.23529412 | 0.235294118 | 4.705882353 |
| 5 | 25 | 0.01 | 0.29411765 | 0.294117647 | 5.882352941 |
| 6 | 30 | 0.011 | 0.32352941 | 0.323529412 | 6.470588235 |
| 7 | 45 | 0.013 | 0.38235294 | 0.382352941 | 7.647058824 |
| 8 | 60 | 0.015 | 0.44117647 | 0.441176471 | 8.823529412 |
| 9 | 90 | 0.018 | 0.52941176 | 0.529411765 | 10.58823529 |
| 10 | 120 | 0.021 | 0.61764706 | 0.617647059 | 12.35294118 |
| 11 | 150 | 0.025 | 0.73529412 | 0.735294118 | 14.70588235 |
| 12 | 180 | 0.03 | 0.88235294 | 0.882352941 | 17.64705882 |
| 13 | 210 | 0.033 | 0.97058824 | 0.970588235 | 19.41176471 |
| 14 | 240 | 0.037 | 1.08823529 | 1.088235294 | 21.76470588 |
| 15 | 270 | 0.04 | 1.17647059 | 1.176470588 | 23.52941176 |
| 16 | 300 | 0.044 | 1.29411765 | 1.294117647 | 25.88235294 |
| 17 | 330 | 0.049 | 1.44117647 | 1.441176471 | 28.82352941 |
| 18 | 360 | 0.052 | 1.52941176 | 1.529411765 | 30.58823529 |

**Table-58**

**Release of F4 formulation microparticles from 1 % w/v carbomer (940) gel**

| S. No. | Time (min.) | Absorbance at 276 nm | Concentration (µg/ml) | Drug released (mg) | % Drug released |
|---|---|---|---|---|---|
| 1 | 5 | 0 | 0 | 0 | 0 |
| 2 | 10 | 0.001 | 0.02941 | 0.02941 | 0.58824 |
| 3 | 15 | 0.002 | 0.05882 | 0.05882 | 1.17647 |
| 4 | 20 | 0.004 | 0.11765 | 0.11765 | 2.35294 |
| 5 | 25 | 0.006 | 0.17647 | 0.17647 | 3.52941 |
| 6 | 30 | 0.009 | 0.26471 | 0.26471 | 5.29412 |
| 7 | 45 | 0.011 | 0.32353 | 0.32353 | 6.47059 |
| 8 | 60 | 0.013 | 0.38235 | 0.38235 | 7.64706 |
| 9 | 90 | 0.015 | 0.44118 | 0.44118 | 8.82353 |
| 10 | 120 | 0.018 | 0.52941 | 0.52941 | 10.5882 |
| 11 | 150 | 0.021 | 0.61765 | 0.61765 | 12.3529 |
| 12 | 180 | 0.024 | 0.70588 | 0.70588 | 14.1176 |
| 13 | 210 | 0.029 | 0.85294 | 0.85294 | 17.0588 |
| 14 | 240 | 0.032 | 0.94118 | 0.94118 | 18.8235 |
| 15 | 270 | 0.035 | 1.02941 | 1.02941 | 20.5882 |
| 16 | 300 | 0.038 | 1.11765 | 1.11765 | 22.3529 |
| 17 | 330 | 0.041 | 1.20588 | 1.20588 | 24.1176 |
| 18 | 360 | 0.045 | 1.32353 | 1.32353 | 26.4706 |

**Table-59**

**Release of F5 formulation microparticles from 0.75 % w/v carbomer (940) gel**

| S. No. | Time (min.) | Absorbance at 257 nm | Concentration (µg/ml) | Drug released (mg) | % Drug released |
|---|---|---|---|---|---|
| 1 | 5 | 0.01 | 0.03125 | 0.03125 | 0.3125 |
| 2 | 10 | 0.014 | 0.09375 | 0.09375 | 0.9375 |
| 3 | 15 | 0.018 | 0.15625 | 0.15625 | 1.5625 |
| 4 | 20 | 0.02 | 0.1875 | 0.1875 | 1.875 |
| 5 | 25 | 0.022 | 0.21875 | 0.21875 | 2.1875 |
| 6 | 30 | 0.025 | 0.265625 | 0.265625 | 2.65625 |
| 7 | 45 | 0.029 | 0.328125 | 0.328125 | 3.28125 |
| 8 | 60 | 0.035 | 0.421875 | 0.421875 | 4.21875 |
| 9 | 90 | 0.044 | 0.5625 | 0.5625 | 5.625 |
| 10 | 120 | 0.05 | 0.65625 | 0.65625 | 6.5625 |
| 11 | 150 | 0.058 | 0.78125 | 0.78125 | 7.8125 |
| 12 | 180 | 0.066 | 0.90625 | 0.90625 | 9.0625 |
| 13 | 210 | 0.078 | 1.09375 | 1.09375 | 10.9375 |
| 14 | 240 | 0.088 | 1.25 | 1.25 | 12.5 |
| 15 | 270 | 0.1 | 1.4375 | 1.4375 | 14.375 |
| 16 | 300 | 0.109 | 1.578125 | 1.578125 | 15.78125 |
| 17 | 330 | 0.123 | 1.796875 | 1.796875 | 17.96875 |
| 18 | 360 | 0.135 | 1.984375 | 1.984375 | 19.84375 |

**Table-60**

**Release of F5 formulation microparticles from 1 % w/v carbomer (940) gel**

| S. No. | Time (min.) | Absorbance at 257 nm | Concentration (µg/ml) | Drug released (mg) | % Drug released |
|---|---|---|---|---|---|
| 1 | 5 | 0.005 | -0.0469 | -0.0469 | -0.4688 |
| 2 | 10 | 0.008 | 0 | 0 | 0 |
| 3 | 15 | 0.011 | 0.04688 | 0.04688 | 0.46875 |
| 4 | 20 | 0.015 | 0.10938 | 0.10938 | 1.09375 |
| 5 | 25 | 0.018 | 0.15625 | 0.15625 | 1.5625 |
| 6 | 30 | 0.021 | 0.20313 | 0.20313 | 2.03125 |
| 7 | 45 | 0.026 | 0.28125 | 0.28125 | 2.8125 |
| 8 | 60 | 0.031 | 0.35938 | 0.35938 | 3.59375 |
| 9 | 90 | 0.035 | 0.42188 | 0.42188 | 4.21875 |
| 10 | 120 | 0.039 | 0.48438 | 0.48438 | 4.84375 |
| 11 | 150 | 0.045 | 0.57813 | 0.57813 | 5.78125 |
| 12 | 180 | 0.052 | 0.6875 | 0.6875 | 6.875 |
| 13 | 210 | 0.061 | 0.82813 | 0.82813 | 8.28125 |
| 14 | 240 | 0.067 | 0.92188 | 0.92188 | 9.21875 |
| 15 | 270 | 0.077 | 1.07813 | 1.07813 | 10.7813 |
| 16 | 300 | 0.091 | 1.29688 | 1.29688 | 12.9688 |
| 17 | 330 | 0.103 | 1.48438 | 1.48438 | 14.8438 |
| 18 | 360 | 0.114 | 1.65625 | 1.65625 | 16.5625 |

**Table-61**

**Release of Diclofenac sodium from F6 formulation microparticles from 0.75 % w/v carbomer (940) gel**

| S. No. | Time (min.) | Absorbance at 276 nm | Concentration (µg/ml) | Drug released (mg) | % Drug released |
|---|---|---|---|---|---|
| 1 | 5 | 0.009 | 0.09677 | 0.09677 | 1.93548 |
| 2 | 10 | 0.012 | 0.14516 | 0.14516 | 2.90323 |
| 3 | 15 | 0.016 | 0.20968 | 0.20968 | 4.19355 |
| 4 | 20 | 0.019 | 0.25806 | 0.25806 | 5.16129 |
| 5 | 25 | 0.022 | 0.30645 | 0.30645 | 6.12903 |
| 6 | 30 | 0.025 | 0.35484 | 0.35484 | 7.09677 |
| 7 | 45 | 0.03 | 0.43548 | 0.43548 | 8.70968 |
| 8 | 60 | 0.036 | 0.53226 | 0.53226 | 10.6452 |
| 9 | 90 | 0.04 | 0.59677 | 0.59677 | 11.9355 |
| 10 | 120 | 0.047 | 0.70968 | 0.70968 | 14.1935 |
| 11 | 150 | 0.055 | 0.83871 | 0.83871 | 16.7742 |
| 12 | 180 | 0.064 | 0.98387 | 0.98387 | 19.6774 |
| 13 | 210 | 0.07 | 1.08065 | 1.08065 | 21.6129 |
| 14 | 240 | 0.079 | 1.22581 | 1.22581 | 24.5161 |
| 15 | 270 | 0.086 | 1.33871 | 1.33871 | 26.7742 |
| 16 | 300 | 0.093 | 1.45161 | 1.45161 | 29.0323 |
| 17 | 330 | 0.101 | 1.58065 | 1.58065 | 31.6129 |
| 18 | 360 | 0.108 | 1.69355 | 1.69355 | 33.871 |

**Table-62**

**Release of Sulfamethoxazole from F6 formulation microparticles from 0.75 % w/v carbomer (940) gel**

| S. No. | Time (min.) | Absorbance at 257 nm | Concentration (µg/ml) | Drug released (mg) | % Drug released |
|---|---|---|---|---|---|
| 1 | 5 | 0.008 | 0.056818182 | 0.0568182 | 0.568181818 |
| 2 | 10 | 0.013 | 0.113636364 | 0.1136364 | 1.136363636 |
| 3 | 15 | 0.019 | 0.181818182 | 0.1818182 | 1.818181818 |
| 4 | 20 | 0.022 | 0.215909091 | 0.2159091 | 2.159090909 |
| 5 | 25 | 0.026 | 0.261363636 | 0.2613636 | 2.613636364 |
| 6 | 30 | 0.031 | 0.318181818 | 0.3181818 | 3.181818182 |
| 7 | 45 | 0.039 | 0.409090909 | 0.4090909 | 4.090909091 |
| 8 | 60 | 0.048 | 0.511363636 | 0.5113636 | 5.113636364 |
| 9 | 90 | 0.059 | 0.636363636 | 0.6363636 | 6.363636364 |
| 10 | 120 | 0.071 | 0.772727273 | 0.7727273 | 7.727272727 |
| 11 | 150 | 0.082 | 0.897727273 | 0.8977273 | 8.977272727 |
| 12 | 180 | 0.096 | 1.056818182 | 1.0568182 | 10.56818182 |
| 13 | 210 | 0.112 | 1.238636364 | 1.2386364 | 12.38636364 |
| 14 | 240 | 0.136 | 1.511363636 | 1.5113636 | 15.11363636 |
| 15 | 270 | 0.169 | 1.886363636 | 1.8863636 | 18.86363636 |
| 16 | 300 | 0.186 | 2.079545455 | 2.0795455 | 20.79545455 |
| 17 | 330 | 0.198 | 2.215909091 | 2.2159091 | 22.15909091 |
| 18 | 360 | 0.221 | 2.477272727 | 2.4772727 | 24.77272727 |

**Table-63**

**Release of Diclofenac sodium from F6 formulation microparticles from 1 % w/v carbomer (940) gel**

| S. No. | Time (min.) | Absorbance at 276 nm | Concentration (µg/ml) | Drug released (mg) | % Drug released |
|---|---|---|---|---|---|
| 1 | 5 | 0.005 | 0.03226 | 0.03226 | 0.64516 |
| 2 | 10 | 0.008 | 0.08065 | 0.08065 | 1.6129 |
| 3 | 15 | 0.011 | 0.12903 | 0.12903 | 2.58065 |
| 4 | 20 | 0.016 | 0.20968 | 0.20968 | 4.19355 |
| 5 | 25 | 0.02 | 0.27419 | 0.27419 | 5.48387 |
| 6 | 30 | 0.023 | 0.32258 | 0.32258 | 6.45161 |
| 7 | 45 | 0.027 | 0.3871 | 0.3871 | 7.74194 |
| 8 | 60 | 0.031 | 0.45161 | 0.45161 | 9.03226 |
| 9 | 90 | 0.036 | 0.53226 | 0.53226 | 10.6452 |
| 10 | 120 | 0.041 | 0.6129 | 0.6129 | 12.2581 |
| 11 | 150 | 0.044 | 0.66129 | 0.66129 | 13.2258 |
| 12 | 180 | 0.054 | 0.82258 | 0.82258 | 16.4516 |
| 13 | 210 | 0.061 | 0.93548 | 0.93548 | 18.7097 |
| 14 | 240 | 0.071 | 1.09677 | 1.09677 | 21.9355 |
| 15 | 270 | 0.077 | 1.19355 | 1.19355 | 23.871 |
| 16 | 300 | 0.082 | 1.27419 | 1.27419 | 25.4839 |
| 17 | 330 | 0.088 | 1.37097 | 1.37097 | 27.4194 |
| 18 | 360 | 0.095 | 1.48387 | 1.48387 | 29.6774 |

**Table-64**

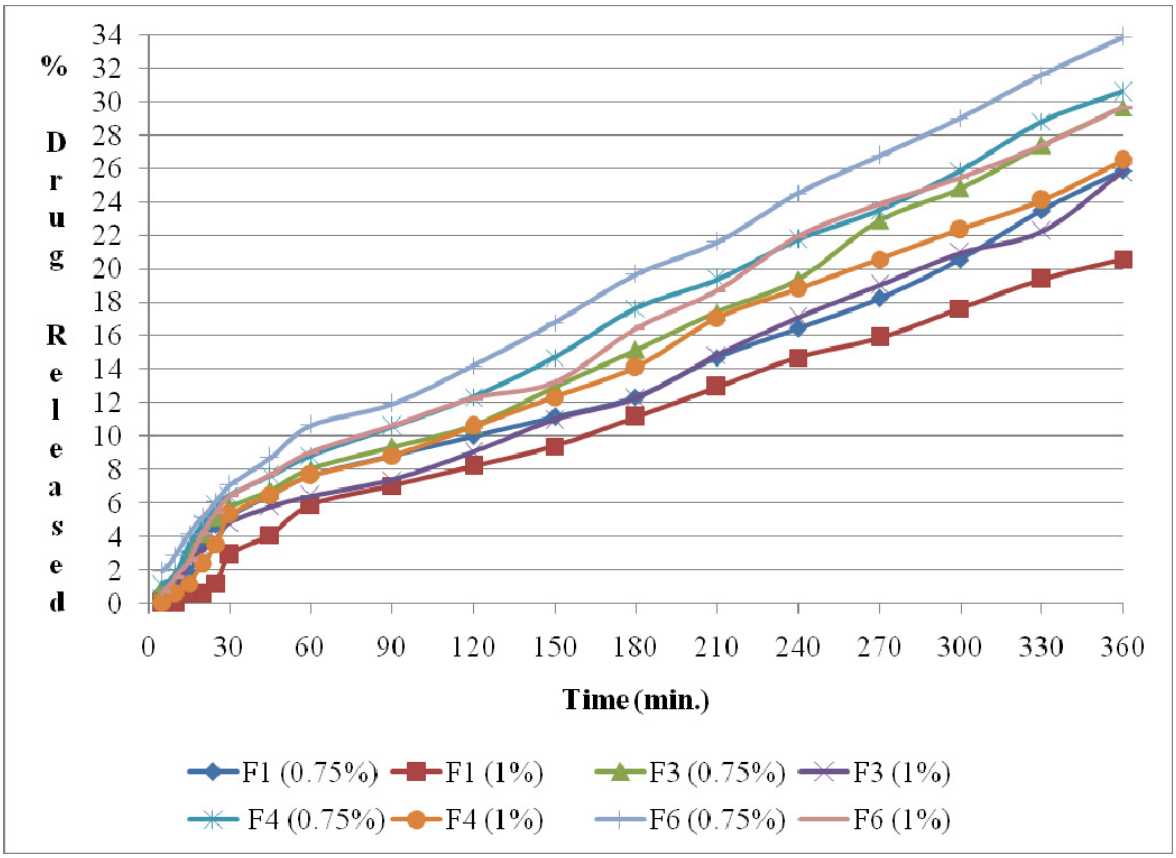
**Release of Sulfamethoxazole from F6 formulation microparticles from 1 % w/v carbomer (940) gel**

| S. No. | Time (min.) | Absorbance at 257 nm | Concentration (µg/ml) | Drug released (mg) | % Drug released |
|---|---|---|---|---|---|
| 1 | 5 | 0.006 | 0.034 | 0.03409 | 0.34091 |
| 2 | 10 | 0.009 | 0.068 | 0.06818 | 0.68182 |
| 3 | 15 | 0.013 | 0.114 | 0.11364 | 1.13636 |
| 4 | 20 | 0.018 | 0.17 | 0.17045 | 1.70455 |
| 5 | 25 | 0.021 | 0.205 | 0.20455 | 2.04545 |
| 6 | 30 | 0.025 | 0.25 | 0.25 | 2.5 |
| 7 | 45 | 0.032 | 0.33 | 0.32955 | 3.29545 |
| 8 | 60 | 0.041 | 0.432 | 0.43182 | 4.31818 |
| 9 | 90 | 0.05 | 0.534 | 0.53409 | 5.34091 |
| 10 | 120 | 0.061 | 0.659 | 0.65909 | 6.59091 |
| 11 | 150 | 0.07 | 0.761 | 0.76136 | 7.61364 |
| 12 | 180 | 0.083 | 0.909 | 0.90909 | 9.09091 |
| 13 | 210 | 0.095 | 1.045 | 1.04545 | 10.4545 |
| 14 | 240 | 0.109 | 1.205 | 1.20455 | 12.0455 |
| 15 | 270 | 0.127 | 1.409 | 1.40909 | 14.0909 |
| 16 | 300 | 0.144 | 1.602 | 1.60227 | 16.0227 |
| 17 | 330 | 0.162 | 1.807 | 1.80682 | 18.0682 |
| 18 | 360 | 0.189 | 2.114 | 2.11364 | 21.1364 |

**Table-65**

**Comparison of Diclofenac sodium microparticles released from two different gel formulations (0.75% and 1% w/v)**

| S. No. | Time (min.) | Percentage drug released | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | F1 (Diclo without chito) | | F3 (Diclo without chito in combi) | | F4 (Diclo with chito) | | F6 (Diclo with chito in combi) | |
| | | 0.75% | 1% | 0.75% | 1% | 0.75% | 1% | 0.75% | 1% |
| 1 | 5 | 0.59 | 0.00 | 0.97 | 0.32 | 1.18 | 0.00 | 1.94 | 0.65 |
| 2 | 10 | 1.18 | 0.00 | 1.61 | 0.97 | 1.76 | 0.59 | 2.90 | 1.61 |
| 3 | 15 | 1.76 | 0.59 | 3.23 | 2.58 | 3.53 | 1.18 | 4.19 | 2.58 |
| 4 | 20 | 3.53 | 0.59 | 4.19 | 3.23 | 4.71 | 2.35 | 5.16 | 4.19 |
| 5 | 25 | 4.71 | 1.18 | 5.16 | 3.87 | 5.88 | 3.53 | 6.13 | 5.48 |
| 6 | 30 | 5.29 | 2.94 | 5.81 | 4.84 | 6.47 | 5.29 | 7.10 | 6.45 |
| 7 | 45 | 6.47 | 4.12 | 6.77 | 5.81 | 7.65 | 6.47 | 8.71 | 7.74 |
| 8 | 60 | 7.65 | 5.88 | 8.06 | 6.45 | 8.82 | 7.65 | 10.65 | 9.03 |
| 9 | 90 | 8.82 | 7.06 | 9.35 | 7.42 | 10.59 | 8.82 | 11.94 | 10.65 |
| 10 | 120 | 10.00 | 8.24 | 10.65 | 9.03 | 12.35 | 10.59 | 14.19 | 12.26 |
| 11 | 150 | 11.18 | 9.41 | 12.90 | 10.97 | 14.71 | 12.35 | 16.77 | 13.23 |
| 12 | 180 | 12.35 | 11.18 | 15.16 | 12.26 | 17.65 | 14.12 | 19.68 | 16.45 |
| 13 | 210 | 14.71 | 12.94 | 17.42 | 14.84 | 19.41 | 17.06 | 21.61 | 18.71 |
| 14 | 240 | 16.47 | 14.71 | 19.35 | 17.10 | 21.76 | 18.82 | 24.52 | 21.94 |
| 15 | 270 | 18.24 | 15.88 | 22.90 | 19.03 | 23.53 | 20.59 | 26.77 | 23.87 |
| 16 | 300 | 20.59 | 17.65 | 24.84 | 20.97 | 25.88 | 22.35 | 29.03 | 25.48 |
| 17 | 330 | 23.53 | 19.41 | 27.42 | 22.26 | 28.82 | 24.12 | 31.61 | 27.42 |
| 18 | 360 | 25.88 | 20.59 | 29.68 | 25.81 | 30.59 | 26.47 | 33.87 | 29.68 |



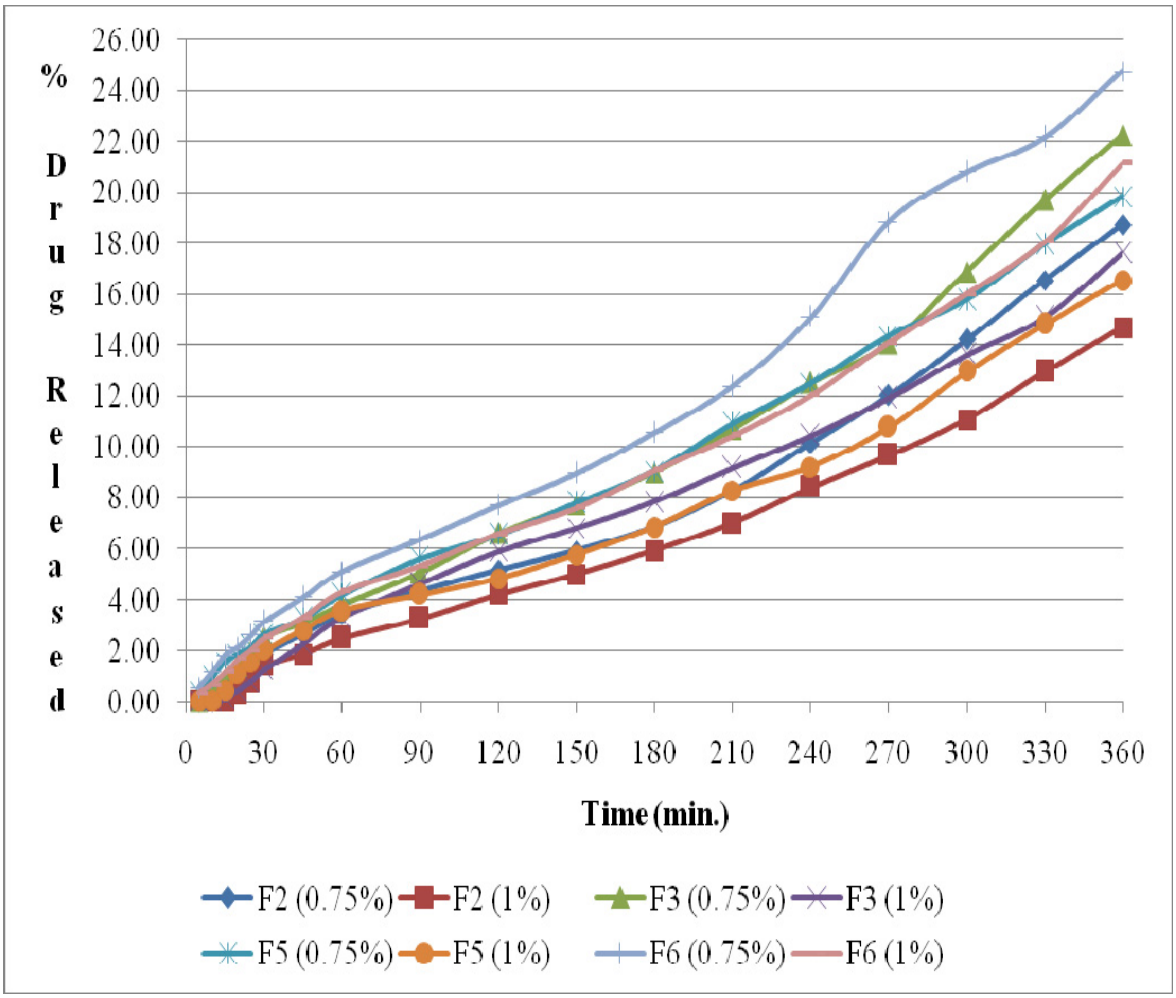

**Graph-24**

**Comparison of Diclofenac sodium microparticles released from two different gel formulations (0.75% and 1% w/v)**

**Table-66**

**Comparison of Sulfamethoxazole microparticles released from two different gel formulations (0.75% and 1% w/v)**

| S. No | Time (min.) | Percentage drug released | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | F2 (Sulfa without chito) | | F3 (Sulfa without chito in combi) | | F5 (Sulfa with chito) | | F6 (Sulfa with chito in combi) | |
| | | F2 (0.75%) | F2 (1%) | F3 (0.75%) | F3 (1%) | F5 (0.75%) | F5 (1%) | F6 (0.75%) | F6 (1%) |
| 1 | 5 | 0.00 | 0.00 | 0.00 | 0.00 | 0.31 | 0.00 | 0.57 | 0.34 |
| 2 | 10 | 0.00 | 0.00 | 0.56 | 0.00 | 0.94 | 0.00 | 1.14 | 0.68 |
| 3 | 15 | 0.16 | 0.00 | 0.84 | 0.11 | 1.56 | 0.47 | 1.82 | 1.14 |
| 4 | 20 | 0.78 | 0.31 | 1.40 | 0.45 | 1.88 | 1.09 | 2.16 | 1.70 |
| 5 | 25 | 1.25 | 0.78 | 1.96 | 0.80 | 2.19 | 1.56 | 2.61 | 2.05 |
| 6 | 30 | 1.88 | 1.41 | 2.53 | 1.25 | 2.66 | 2.03 | 3.18 | 2.50 |
| 7 | 45 | 2.66 | 1.88 | 3.09 | 2.27 | 3.28 | 2.81 | 4.09 | 3.30 |
| 8 | 60 | 3.44 | 2.50 | 3.79 | 3.30 | 4.22 | 3.59 | 5.11 | 4.32 |
| 9 | 90 | 4.38 | 3.28 | 5.06 | 4.66 | 5.63 | 4.22 | 6.36 | 5.34 |
| 10 | 120 | 5.16 | 4.22 | 6.61 | 5.91 | 6.56 | 4.84 | 7.73 | 6.59 |
| 11 | 150 | 5.94 | 5.00 | 7.74 | 6.82 | 7.81 | 5.78 | 8.98 | 7.61 |
| 12 | 180 | 6.88 | 5.94 | 9.01 | 7.84 | 9.06 | 6.88 | 10.57 | 9.09 |
| 13 | 210 | 8.28 | 7.03 | 10.70 | 9.20 | 10.94 | 8.28 | 12.39 | 10.45 |
| 14 | 240 | 10.16 | 8.44 | 12.53 | 10.45 | 12.50 | 9.22 | 15.11 | 12.05 |
| 15 | 270 | 12.03 | 9.69 | 14.08 | 11.93 | 14.38 | 10.78 | 18.86 | 14.09 |
| 16 | 300 | 14.22 | 11.09 | 16.89 | 13.64 | 15.78 | 12.97 | 20.80 | 16.02 |
| 17 | 330 | 16.56 | 12.97 | 19.71 | 15.11 | 17.97 | 14.84 | 22.16 | 18.07 |
| 18 | 360 | 18.75 | 14.69 | 22.25 | 17.61 | 19.84 | 16.56 | 24.77 | 21.14 |




**Graph-25**

**Comparison of Sulfamethoxazole microparticles released from two different gel formulations (0.75% and 1% w/v)**

**Corollary:**

F1 formulation in 0.75 % w/v carbomer (940) gel 25.88 % of drug was released for a period of 6 hrs. F1 formulation in 1 % w/v carbomer (940) gel 20.59 % of drug was released for a period of 6 hrs. F2 formulation in 0.75 % w/v carbomer (940) gel 18.75 % of drug was released for a period of 6 hrs. F2 formulation in 1 % w/v carbomer (940) gel 14.69 % of drug was released for a period of 6 hrs. Release of Diclofenac sodium from F3 formulation in 0.75 % w/v carbomer (940) gel 29.68 % of drug was released for a period of 6 hrs. Release of Sulfamethoxazole from F3 formulation in 0.75 % w/v carbomer (940) gel 22.25 % of drug was released for a period of 6 hrs. Release of Diclofenac sodium from F3 formulation in 1 % w/v

carbomer (940) gel 25.81 % of drug was released for a period of 6 hrs. Release of Sulfamethoxazole from F3 formulation in 1 % w/v carbomer (940) gel 17.61% of drug was released for a period of 6 hrs. F4 formulation in 0.75 % w/v carbomer (940) gel 30.59 % of drug was released for a period of 6 hrs. F4 formulation in 1 % w/v carbomer (940) gel 26.47 % of drug was released for a period of 6 hrs. F5 formulation in 0.75 % w/v carbomer (940) gel 19.84 % of drug was released for a period of 6 hrs. F5 formulation in 1 % w/v carbomer (940) gel 16.56 % of drug was released for a period of 6 hrs. Release of Diclofenac sodium from F6 formulation in 0.75 % w/v carbomer (940) gel 33.87 % of drug was released for a period of 6 hrs. Release of Sulfamethoxazole from F3 formulation in 0.75 % w/v carbomer (940) gel 24.77 % of drug was released for a period of 6 hrs. Release of Diclofenac sodium from F6 formulation in 1 % w/v carbomer (940) gel 29.68 % of drug was released for a period of 6 hrs. Release of Sulfamethoxazole from F3 formulation in 1 % w/v carbomer (940) gel 21.13 % of drug was released for a period of 6 hrs.

**Release Kinetics**

The drug released data was fitted to various release kinetics. The formulation follows zero order kinetics. The results were shown in table-67

**Table-67**
**Drug release kinetics of microparticles formulations**

| Formulations | Concentrations (%w/v) | Zero order | First order | Higuchi | Hixson | Korsmeyer-Peppas | n value |
|---|---|---|---|---|---|---|---|
| **F1 (Diclo without chito** | **0.75%** | 0.978 | 0.978 | 0.96 | 0.978 | 0.954 | y = 0.777x - 0.588 |
| | **1%** | 0.985 | 0.989 | 0.984 | 0.989 | 0.902 | y = 0.898x - 0.978 |
| **F2 (Sulfa without chito** | **0.75%** | 0.977 | 0.97 | 0.919 | 0.974 | 0.83 | y = 0.876x - 1.08 |
| | **1%** | 0.988 | 0.985 | 0.938 | 0.987 | 0.846 | y = 0.781x - 0.964 |
| **F3 (Diclo without chito in combi)** | **0.75%** | 0.99 | 0.988 | 0.961 | 0.988 | 0.975 | y = 0.722x - 0.404 |
| | **1%** | 0.989 | 0.987 | 0.962 | 0.988 | 0.937 | y = 0.839x - 0.736 |
| **F3 (Sulfa without chito in combi)** | **0.75%** | 0.985 | 0.977 | 0.929 | 0.981 | 0.954 | y = 0.842x - 0.902 |
| | **1%** | 0.993 | 0.992 | 0.961 | 0.993 | 0.802 | y = 0.955x - 1.265 |
| **F4 (Diclo with chito)** | **0.75%** | 0.989 | 0.993 | 0.979 | 0.99 | 0.977 | y = 0.704x - 0.326 |
| | **1%** | 0.0983 | 0.989 | 0.984 | 0.987 | 0.941 | y = 0.854x - 0.742 |
| **F5 (Sulfa with chito)** | **0.75%** | 0.994 | 0.991 | 0.954 | 0.993 | 0.982 | y = 0.848x - 0.902 |
| | **1%** | 0.982 | 0.977 | 0.935 | 0.98 | 0.918 | y = 0.767x - 0.847 |
| **F6 (Diclo with chito in combi)** | **0.75%** | 0.991 | 0.995 | 0.982 | 0.991 | 0.994 | y = 0.645x - 0.145 |
| | **1%** | 0.984 | 0.989 | 0.978 | 0.986 | 0.959 | y = 0.778x - 0.497 |
| **F6 (Sulfa with chito in combi)** | **0.75%** | 0.988 | 0.981 | 0.934 | 0.985 | 0.99 | y = 0.820x - 0.759 |
| | **1%** | 0.988 | 0.982 | 0.94 | 0.985 | 0.989 | y = 0.886x - 0.993 |

# *Conclusions*

# CONCLUSION

The presence of more than one API in single-unit dosage form such as tablet is common practice and even had the market success. However, development of multiple-unit dosage form such as microparticles with more than one API is not a common practice and none of the microparticles entered so far into market using the concept of dual drug loading. In this work, the two drugs namely, Diclofenac sodium and Sulfamethoxazole were encapsulated into microparticles formulation prepared by cold/hot-dispersion method. But after the microparticles formation, the current pilot experimental studies using IR spectroscopy, UV-analysis, TLC, etc. indicated that both of these two APIs underwent interaction within the microparticles itself. Thus further experimental studies should be needed to confirm the fact that whether or not the predicted interaction between these two drugs occurred at molecular (solid solution) level inside the hydrophilic polymer-based microparticles.

# References

# BIBLIOGRAPHY

1. B. Pavan Kumar, I. Sarath Chandiran, B. Bhavya and M. Sindhuri. Microparticle drug delivery system. Indian Journal of Pharmaceutical Science & Research, 2011; 1(1): 19-37.
2. N.V. A.Lamprecht, H.TRodero, U.Schafer and C.LMichael, Biodegradable icroparticles as a two-drug controlled release formulation: a potential treatment of nflammatory bowel disease Journal of Controlled Release 2000; 69: 445–454.
3. K,Tagawa , M Kawata M and S. Goto Biopharmaceutical evaluation of gelatine microcapsules of several antibiotics. . Journal Pharmaceutical Science & Research, 1983; 31: 269–273.
4. S,Goto, M Komatsu, K Tagawa and M Kawata, Preparation and evaluation of gelatin microcapsules of sulfonamides. Journal Pharmaceutical Science & Research,1983; 31: 256–261.
5. M Komatsu, K Tagawa K, M Kawata and Goto S. Biopharmaceutical evaluation of gelatin microcapsules of sulfonamides. Journal Pharmacetical Science & Research, 1983; 31: 262–268.
6. J R Nixon and M R Meleka, The in vivo performance of theophylline microcapsules, Journal Microencapsulation, 1999; 1: 65–72.
7. Tikesh Kumar Banothe, Siddharth Kumar, Ajazuddin, Amit Alexander, D. K. Tripathi. International Journal of Pharmaceutics, 2000; 1(2): 79-86.

8. Madan P L, Jani R K and Bartilucci A J, New method of preparing gelatine microcapsules of soluble pharmaceuticals. Journal Pharmaceutical Science and Research, 1978; 67: 409–411.
9. Nixon J R, Khalil S A H and Carless J E. Gelatin coacervate microcapsules containing Sulpham erazine: their preparationand the invitro release of drug. Journal Pharmacy and pharmacology, 1968; 20: 528–538.
10. S .A. H, Nixon J .R and Carless JE. Role of pH in the coacervation of the systems: gelatine–water– ethanol and gelatine–water– sodium sulphate. Journal Pharmacy and Pharmacology, 1968; 20: 215–225.
11. S.Goto, M.Komatsu, K.Tagawa and M.Kawata, Preparation and evaluation of gelatin Microcapsules of sulfonamides. Journal Pharmaceutcal science and Research, 1983; 31: 256 – 261.
12. H. K. Sah, R Toddywala and Y. W. Chien. Biodegradable microcapsules prepared by a w/o/w technique: effects of shear force to make a primary w/o emulsion on their morphology and protein release. Journal Microencapsules, 1995; 12: 59–69.
13. R,Nagendra Charan CS, K. Hanumanthachar Joshi and C.Jayanthi. Preparation and evaluation of microparticles containing Oxybutynin Chloride for controlled release. Asian Journal of Biomedical and Pharmaceutical Science, 2015; 5(41):15-17.
14. Jagadeesh Nadigoti, Sathish Dharani, Shayeda and Madhusudan Rao Yamsani. Formulation and evaluation of floating

microparticles of metoprolol succinate. Asian Jounal Pharmmaceutical Clinical Research, 2011; 4: 132-135.

15. Mitra Jelvehgari, Fatemeh Khonsari and Parvin Zakeri-Milani. Formulation and Evaluation of In-vitro Characterization of Gastic-Mucoadhesive Microparticles / Discs Containing Metformin Hydrochloride. Iran Journal Pharmaceutical Research, 2014; 13(1): 67–80.
16. P.Veera Lakshmi, KPR Chowdary, A. Prameela Rani S and VUM Prasad. Preparation and Evaluation of Micro particulate Drug Delivery Systems of Gliclazide Employing Starch Acetate. Drug Designing & Intellectual Properties International jounal research article, 2018; 1(1): 1-5.
17. Josephine Leno Jenita. Formulation and evaluation of microparticles containing curcumin for colorectal cancer. Journal of Drug Delivery & Therapeutics Research article; 2012; 2(3): 125-128.
18. DV Gowda, MP Gowrav, HV Gangadharappa and MS Khan. Preparation and evaluation of mixture of eudragit and ethylcellulose microparticles loaded with ranolazine for controlled release. Journal young Pharmaceutical reserach article , 2011; 3(3): 189–196.
19. V. Pai Rohan, R. Jain Rajesh, Bannalikar, S. Anil Kunmar and Menon Mala D. Development and evaluation of chitosan microparticles based dry powder inhalation formulations of rifampicin and rifabutin. Jounal Aerosol pumbed drug delivery, 2016; 29(2): 179-195.
20. Balagani. Pavan Kumar, Irisappan, Sarath Chandiran, Korlakunta. Narasimha. Jayaveera. Formulation and evaluation of glimepiride

loaded cellulose acetate microparticles. International Journal of Pharmacy and Pharmaceutical Sciences, 2014; 6(4): 511-515.

21. ZhiYong Qian, XingYi Li, XiangYe Kong, Shuai Shi, Xiu Ling Zheng, Gang Guo and Yu Quan Wei. Preparation of alginate coated chitosan microparticles for vaccine delivery. BMC Biotechnology, 2008; 8:89: 1-13.
22. MajaSimonoska Crcarevska, MarijaGlavas Dodov and KaterinaGoracinova. Done a chitosan coated ca–alginate microparticles loaded with budesonide for delivery to the inflamed colonic mucosa. European Journal of Pharmaceutics and Biopharmaceutics**,** 2008; 68( 3): 565-578.
23. S.Nahla Barakat and S.Alanood Almurshedi. Design and development of gliclazide loaded chitosan microparticles for oral sustained drug delivery. invitro/invivo evaluation. Journal of pharmacy, 2011; 63(2): 169-178.
24. S.Ahmed Zidan, A.Omaima Sammour, A.Mohammed Hammad, A.Nagia Megrab, D. Muhammad Hussain, A.Manosor Khan and J. Muhammad Habib. Formulation of anastrozole microparticles as biodegradable anticancer drug carriers. Pharmma ceutical Science Technology, 2006; 7: 38–46.
25. M.Hombreiro Perez, C .Zinutti, A.Lamprecht, N.Ubrich, M.Hoffman, R.Bodmeier and Maincent P. The preparation and evaluation of poly(ϵ-caprolactone) microparticles containing both a lipophilic and a hydrophilic drug. Journal of Controlled Release, 2000; 65(3): 429-438.
26. Sakchai Wittaya-areekul, Jittiporn Kruenate and Chureerat Prahsarn. Preparation and invitro evaluation of mucoadhesive properties of alginate/chitosan microparticles containing

prednisolone. International Journal of Pharmaceutics, 2006; 312: 113-118.

27. Manmohan Singh, Maylene Briones, Gary Ott and Derek O'Hagan. Cationic microparticles: A potent delivery system for DNA vaccines. Proceedings of a national acedemy of sciences article. 2000; 97(2): 811-816.
28. Philip F.Builders, Olobayo O.Kunle, Larry C.Okpaku, Modupe I.Builders, A Anthony. Attama and U Michael. Adikwu. Preparation and evaluation of mucinated sodium alginate microparticles for oral delivery of insulin. European Journal of Pharmaceutics and Biopharmaceutics, 2008; 70(3): 777-783.
29. Jianhua Zheng , Chaowu Liu , Decai Bao, Yanjun Zhao and Xiaojun Ma. Preparation and evaluation of floating-bioadhesive microparticles containing clarithromycin for the eradication of Helicobacter pylori. Journal Applcaton Polym Science, 2006; 102: 2226–2232.
30. I. Mvander Lubben, J.C. Verhoef, A. Cvan Aelst, G. Borchard and H.E. Junginger. Chitosan microparticles for oral vaccination: Preparation, characterization and preliminary in vivo uptake studies in murine Peyer's patches.(Journal of drug targeting) science direct article 2001; 22(7): 687-694.
31. Mcuna M.J and Alonso D Torres. Preparation and in vivo evaluation of mucoadhesive microparticles containing amoxycillin–resin complexes for drug delivery to the gastric mucosa. European Journal of Pharmaceutics and Biopharmaceutics, 2001; 51(3): 199-205.
32. J.A. Ko , H.J. Park , S.J. Hwang, J.B. Park and J.S. Lee. Preparation and characterization of chitosan microparticles

intended for controlled drug delivery. International Journal of Pharmaceutics, 2002; 249(1–2): 165-174.

33. R.P.Raffin, L.M.ColomeA, R.Pohlmann and S.S.Guterres. Preparation, characterization, and in vivo anti-ulcer evaluation of pantoprazole-loaded microparticles. European Journal of Pharmaceutics and Biopharmaceutics , June 2006; 63,2: 198-204.
34. Herbert Hoyer ,Wolfgang Schlocker ,KafedjiiskiKrum and AndreasBernkop -Schnürch. Preparation and evaluation of microparticles from thiolated polymers via air jet milling. European Journal of Pharmaceutics and Biopharmaceutics, 2008;69, 2: 476-485.
35. NahedHegazy, MüzeyyenDemirel and YaseminYazan. Preparation and in vitro evaluation of pyridostigmine bromide microparticles. International Journal of Pharmaceutics, 2002, 242, 1–2: 171-174.
36. Behzad Sharif Makhmal Zadeh , Fatemeh Moshtaghi , Fakher Rahim and Abbas akhgari. preparation and evaluation of sodium diclofenac loaded chitosan controlled release microparticles using factorial design. International Journal of Drug Development and Research, 2010; 55, 1:131-136.
37. R.P.Raffin, L.M.ColomeA, R.Pohlmann and S.S.Guterres. Preparation, characterization, and in vivo anti-ulcer evaluation of pantoprazole-loaded microparticles. European Journal of Pharmaceutics and Biopharmaceutics , June 2006; 63(2): 198-204.
38. G.C.BazzoE,LemosSenna A.Tand N.Pires. Polyhydroxybutyrate) /chitosan/ ketoprofen or piroxicam composite microparticles:

Preparation and controlled drug release evaluation. Science direct article, 2009; 77, 4: 839-844.

39. NahedHegazy, MüzeyyenDemirel and YaseminYazan. Preparation and in vitro evaluation of pyridostigmine bromide microparticles. International Journal of Pharmaceutics, 2002; 242,1–2: 171-174.
40. KC. Ofokansi1, FC .Kenechukwu1, AB. Isah and EL Okigbo. Formulation and Evaluation of Glutaraldehyde-Crosslinked Chitosan Microparticles for the Delivery of Ibuprofen. Tropical Journal of Pharmaceutical Research, February 2013, ISSN: 1596-5996, 12 (1): 19-25.
41. o .Rania. Salama, Daniela Traini, Hak-KimChan,Adrian Sung, J Alaina. Ammit and Paul M. Young. Preparation and Evaluation of Controlled Release Microparticles for Respiratory Protein Therapy.Jounral pharmaceutical science, 2009; 98, 8: 209-21.
42. Priscileila Colerato Ferrari, Marina Penteado de Almeida and Lais Nohemann. Floating ability and drug release evaluation of gastro retentive microparticles system containing metronidazole obtained by spray drying. Jounral pharmaceutical science, 2017; 53: 1.
43. https://pubmed chemistry. ncbi.nlm.nih.gov/ compound/ Diclofenac_sodium
44. PD. Fowler. Voltarol: diclofenac sodium. Clinical Rheumatic Disease. 1979; 5:427-64
45. RN.Brogden, RC.Heel and GE.Pakes, Diclofenac sodium: a review of its pharmacological properties and therapeutic use in rheumatic diseases and pain of varying origin. Drugs. 1980; 20: 24-48.

46. https://en.wikipedia.org/wiki/Diclofenac.
47. PA.Todd, EM.Sorkin. Diclofenac sodium: a reappraisal of its pharmacodynamic and pharmacokinetic properties, therapeutic efficacy. Drugs. 1988; 35:244-85.
48. Hart FD, Huskisson EC, Answell BM. Non-steroidal anti-inflammatory analgesics. In: Hart FD, ed. Drug treatment of the rheumatic diseases. 2nd ed. Balgowlah, NSW Australia:ADIS Press; 1982:7-60.
49. Goodman Gilman's, The Pharmacological Basis of therapeutics. 1995;11:671-754.
50. https://www.medindia.net/drug-price/diclofenac.htm.
51. https://www.drugbank.ca/salts/DBSALT000466
52. https://pubmed chemistry.ncbi.nlm.nih.gov/compound/sulfamethoxazole
53. Françoise van Bambeke, Paul M. Tulkens, infectious diseases,2017;4:234-238.
54. Environmental Toxicology and Pharmacology, September 2016; 46: 45-54
55. Ramya Krishna Seelam, and Eliyas Kadi Abafita1Journal Pharmaceutical Science , 2012, 101(10): 3787-3798.
56. http://www.rxwiki.com/sulfamethoxazole
57. www.sciencedirect.com/topics/neuroscience/sulfonamides
58. http://www.empr.com/bactrim/drug/1720/
59. www.drugs.com/ingredient/sulfamethoxazole.html
60. " Hand book of Pharmaceutical excipients ". Raymond C.R, Paul J.S, and Sian C.O. 5th Ed Pharmaceutical Association, Pharmaceutical Press;Washington;2006:120,132,430.

61. Anonymous. US warning on HIV drug excipient. International Journal Pharmacy 2000;264:685.
62. G Martin, L Finberg. Propylene glycol: a potentially toxic vehicle inliquid dosage form. Journal Pediatrics 1970; 77: 877–878.
63. **"**Hand book of Pharmaceutcal excipients ''. H Lessmann et al. Skin-sensitizing and irritant properties of propylene glycol. Contact Dermatitis 2005; 53(5): 247–259.
64. N Garti, O. Arkad Preparation of cloudy coconut oil emulsions containing dispersed TiO2 using atomizer. Journal Dispersion Science Technology1986; 7(5): 513–523.
65. HW Nielsen et al. Intranasal administration of different liquid formulations of bumetanide to rabbits. International Journal Pharmacy 2000; 204(1–2): 35–41.
66. C Viseras et al. Uses of clay minerals in semisolid health care and therapeutic product Application Clay Science 2007; 36(1-3): 37–50.
67. JE Browne et al. Characterization and adsorptive properties of pharmaceutical grade clays. Journal Pharmaceutical Science 1990; 69(7): 816–823.
68. SB Ruddy et al. X-ray contrast compositions containing iodoaniline derivatives and pharmaceutically acceptable clays. United States Patent No. 51995;32(1-2):424.
69. GM.Khan, Z. Jiabi Formulation and in vitro evaluation of ibuprofen-Carbopol 974P-NF controlled release matrix tablets III: influence of coexcipients on release rate of the drug.Journal Control Release 1998; 54(2): 185–190.
70. JO. Carnali, MS. Naser. The use of dilute solution viscosity to characterize the network propertiesof carbopol microgels. Colloid

olym Science 1992; 270: 183–193.Amin PD et al Studies on gel tears.

71. A.Taylor, A.Bagley Tailoring closely packed gel-particles systems for use as thickening agents. Journal Application Polymer Science 2002; 21: 113–122.
72. Herbert A. Lieberman, Leon Lachman and Joseph B. Schwartz. Pharmaceutical dosage forms Tablets. 2nd edition, Marcel Dekker, Inc. Dekker New York, 1990;7:123-126.
73. Vikas Parashar, Dabeer Ahmad Surya Prakash Gupta, Neeraj Upmanyu, Neha Parashar. formulation and evalution of biodegradable microspheres of Tinidazole. International Journal of Drug Delivery; 2010;2: 238-241.
74. .S.S.Bansode, S.K.Banarjee, D.D.Gaikwad Microencapsulation: a review. Asian Journal of Pharmaceuties; 2010;1(2):38-39.
75. Indian pharmacopoeiaVol I,Govt of India, Ministry of health and family welfare,1996; 4:477-478.
76. Y.Baimark, and P. Srihanam. Effect of methonal treatment on regenerated silk fibroin microparticles prepared by the emulsification-diffusion technique. Journal Applied Science, 2009;4:3876 - 3881.
77. A.Vyadav, A.Shete, S.Dabkecoden (USA).A.P. Formulation and In-vitro Evalution of Aceclofenac Microcapsules. IJPRIF; 2009;1(2):135-138.
78. Indian pharmacopoeia, Govt Of India, Ministry of Health and Family Welfare. New Delhi: 1996; 2:668-673,735-736.
79. S Mutalik, .A Naha, P.Anju, A.k Ranjith, P.Musmade, K.Manoj and M.Prasanna. Preparation, in vitro, preclinical and clinical

evalution of once daily sustained release tables of aceclofenac. Arch Pharmaceutcal Research,2007;30:222-234.

80. C.V.S. Subrahmanyam Laboratory Manual of physical pharmaceutics,2013;(2):102-107.
81. United States Pharmacopoeia and National Formulary, United States Pharmacopoeial Convention,Inc;24th Ed : Rockville: MD,2000; 2051-2052.
82. S.D. Barhate, K.R.Bavaskar, Y.S.Saroji, M.Potdar, T.N.Gholap, 2009. Development of transdermal dug delivey sytem of ketopofen. IJPRD. Vol.1 (10); 1-7.
83. Y.S. tanwar., formulation and evalution of topical diclofenac sodium gel using different gelling agent, AJPRHC,2007;Vol 4: 1
84. J.Hadgraft, M.LANE, Skin permeation: the years of enlightenment. International Journal Pharmaceutics,2005;2-12:305.
85. Raja Sekharan T, Palanichamy S, Shanmuganathan S, Karthikeyan A, Senthilkumar S.R and Thanga thirupathi A. Formulation and evalution of Microparticle prepared by chitosan, ARS Parmaceutica,2009;50(4):205-214.